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Habitat Partitioning and Associated Morphological Differences Among Three Species of Catostomidae (Teleostei: Actinopterygii) in the South Fork Roanoke River, Virginia

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ABSTRACT

The upper Roanoke River has 11 species of Catostomidae including *Moxostoma ariommum,* Bigeye Jumprock; *Moxostoma cervinum,* Blacktip Jumprock; and *Thoburnia rhothoeca,* Torrent Sucker. Resource partitioning appears to be a key component of maintaining diverse fish assemblages with habitat and food partitioning cited as especially important in communities containing members of the same family. The diets of these species have been documented in previous work revealing only modest differences among them. Snorkeling observations and subsequent quantification of microhabitat were conducted to illuminate habitat partitioning among these morphologically and ecologically similar species. *Thoburnia rhothoeca* inhabited the shallowest, fastest water, over the smallest substrate, and *Moxostoma ariommum* inhabited the deepest, slowest water, over the largest substrate, with *M. cervinum* intermediate for all habitat variables. In an effort to correlate morphological adaptations to these different microhabitats, 22 body measurements were included in a Principal Component Analysis revealing a bigger eye for *M. ariommum* and more fusiform bodies for *T. rhothoeca* and *M. cervinum* consistent with findings in other species inhabiting faster waters. Other correlations among morphology and microhabitat were less clear.

Keywords: *Moxostoma, Thoburnia,* suckers, snorkel observations

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INTRODUCTION

The upper Roanoke River has 11 species of Catostomidae including several highly range restricted species (Bugas et al., 2019). This diverse fauna includes *Moxostoma ariommum,* Bigeye Jumprock; *Moxostoma cervinum,* Blacktip Jumprock; and *Thoburnia rhothoeca,* Torrent Sucker. These species are all less than 200 mm standard length and restricted to small or medium sized high to moderate gradient streams of the central Atlantic slope. They can also be found within the same stream reaches in the upper Roanoke (see Jenkins and Burkhead, 1994 for dot distribution maps) in close proximity to one another including the same seine haul (S.L. Powers, personal observation). In addition to having similar ranges and habitats, other ecological similarities have been documented for these species (Jenkins and Burkhead, 1994; Tarasidis and Powers, 2014; Thompson et al., 2015)

Resource partitioning appears to be a key component of maintaining diverse fish assemblages, with habitat and food partitioning cited as especially important in communities containing members of the same family (Ross, 1986). The diets of these species have been documented in previous work with *T. rhothoeca* feeding mostly on detritus and Chironomidae, but also feeding on other Diptera as well as Ephemeroptera and Trichoptera (Tarasidis and Powers, 2014). *Moxostoma cervinum* feed largely on Chironomidae, while also ingesting Ephemeroptera, Trichoptera, and Acari (Thompson et al., 2015). *Moxostoma ariommum* have a varied diet including Ephemeroptera, Trichoptera, and other Diptera in addition to large numbers of Chironomidae (Jenkins and Burkhead, 1994). As these differences in diet are modest, it is unlikely they alone are responsible for the resource partitioning allowing the coexistence of these species in small streams like the South Fork Roanoke River. Habitat partitioning has been cited as especially important in structuring stream fish assemblages (Grossman and Freeman, 1987, Grossman et al., 1998).

While the diets of these species have been documented in previous studies, detailed investigations into their specific microhabitats are largely lacking. Additionally, investigations of morphological adaptation to specific microhabitats facilitating this partitioning is completely lacking for these species. Morphological changes associated with specific microhabitats have been documented for other species, but appears to be a complex process with all interactions of morphology and microhabitats occurring within the context of selection on the entire organism leaving specific interactions of morphology and microhabitat challenging to elucidate (Domenici, 2003; Langerhans et al., 2007). This suggests precise microhabitat data accompanied by precise morphological data are necessary to tease apart partitioning that allows coexistence of these ecologically and morphologically similar syntopic species. Therefore, the objective of this study was to examine the microhabitat and morphology of each species for evidence of resource partitioning and morphological adaptation to their specific microhabitats.

METHODS

Snorkeling observations occurred from 2018 to 2020 in June and July to quantify microhabitat of each species. The study site was in the South Fork Roanoke River in Montgomery County, VA 7.6 km SSE of Shawsville upstream of Allegheny Springs Road near Camp Alta Mons

(37° 06' 03.04" N, 80° 14' 59.29" W). At this locality, the stream is less than 15 m wide with maximum depth less than 1.5 m at normal summer flows. All observations were made with flows between 50 and 100 cubic feet per second on the stream flow gauge (Station Number 02053800) maintained by the United States Geologic Survey on the South Fork Roanoke River near Shawsville, VA (https://waterdata.usgs.gov/va/nwis/current/?type=flow). In addition to avoiding high flows, periods of high turbidity were also avoided for snorkeling due to difficulty in observation of study species and subsequent data collection.

Observation and subsequent habitat data collection followed Spruill and Powers (2019). Snorkeling observations were made moving downstream. Data were collected for all three species on the same days. At the point of first sighting of an individual of each target species, a 5 cm diameter galvanized steel marker numbered and painted fluorescent green was placed on the substrate. At each marker, water depth and the diameter of five representative rocks within 10 cm of the marker were measured with a meter stick. Bedrock greater than 1 m across was recorded as 100 cm. The identification of the target species and all habitat data were recorded on a dive slate. Current velocity approximately 5 cm above the substrate was measured with a JDC Electronics FloWatch FW450 flowmeter. For depth and velocity, a total of 30, 35, and 36 observations were made for *M. ariommum, M. cervinum,* and *T. rhothoeca,* respectively. For substrate, the diameters of the five representative rocks at each observation point were summed and divided by five to find the mean value for substrate diameter. That mean value for each observation point was used for analyses. No attempt was made to quantify all available habitat. Equal variances were not assumed for habitat data, therefore a Welch's one-way analysis of variance (ANOVA) was conducted to test for differences in occupied habitat among species for each measured habitat variable with alpha levels of 0.05. Minitab 19 (Minitab LLC. State College, Pennsylvania) was used to calculate descriptive statistics, statistical analyses, and generate interval plots.

Twenty-two measurements were taken using Fowler Pro Max (No. 614624) digital calipers from specimens (n = 10) of each species to quantify differences in body shape (Fig. 1). Fineness ratio was calculated for each specimen by dividing standard length (SL) by the body depth at the dorsal fin origin. Fineness ratios among species were compared with a Welch's ANOVA. Following Armbruster and Page (1996), the 22 raw measurements were natural-log transformed in Excel 2016, and a principal component (PC) analysis of those transformed data was performed in Minitab 19 to quantify differences in shape of the species. Size variation of specimens was accounted for in PC 1, leaving PC 2 and PC 3 as functions of shape differences. Scatterplots of PC 2 and PC 3 scores were examined for morphological differences among the species. Body measurements with absolute Eigenvector values of > 0.3 were considered especially influential on PC scores and are reported after the measurements in Results.

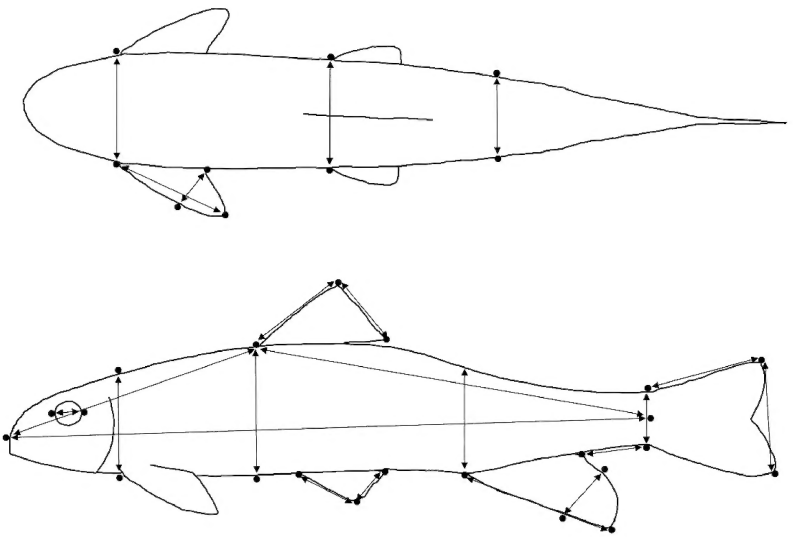

FIGURE 1: Twenty-two measurements taken from *Moxostoma ariommum*, *M. cervinum*, and *Thoburnia rhothoeca* for the principal component analysis quantifying morphological differences among species.

RESULTS

Current velocity was not equal among species ($P < 0.001$; mean for *Moxostoma ariommum* = 0.18 m/s, SE = 0.02; mean for *Moxostoma cervinum* = 0.25 m/s, SE = 0.02; mean for *Thoburnia rhothoeca* = 0.54 m/s, SE = 0.03; Fig. 2). Depth of water observed in was not equal among species ($P < 0.001$; mean for *Moxostoma ariommum* = 85.93 cm, SE = 3.67; mean for *Moxostoma cervinum* = 56.51 cm, SE = 2.22; mean for *Thoburnia rhothoeca* = 39.4 cm, SE = 1.28; Fig. 3). Substrate size also was not equal among species ($P < 0.001$; mean for *Moxostoma ariommum* = 39.07 cm, SE = 5.71; mean for *Moxostoma cervinum* = 14.95 cm, SE = 1.66; mean for *Thoburnia rhothoeca* = 9.9 cm, SE = 0.69; Fig. 4).

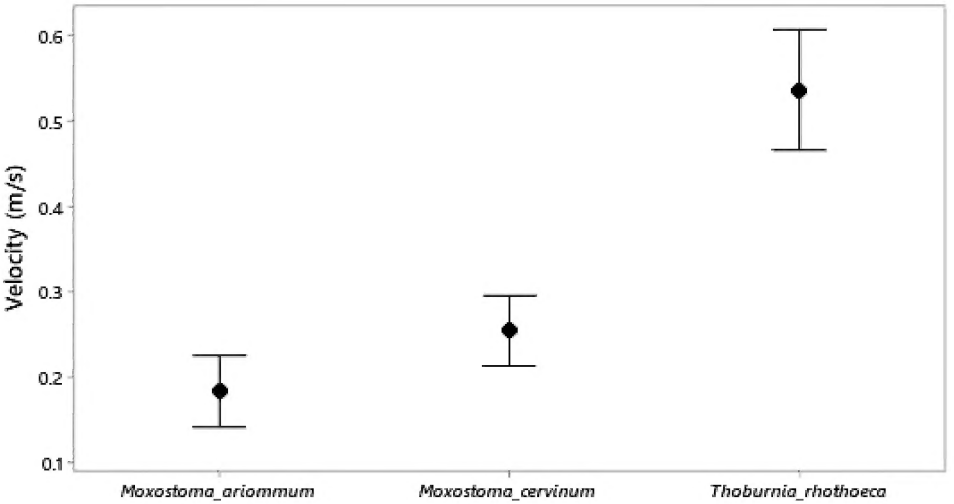

FIGURE 2: Plot of 95% confidence intervals of mean velocity of water occupied for *Moxostoma ariommum* (n = 30), *M. cervinum* (n = 35), and *Thoburnia rhothoeca* (n = 36) in the South Fork Roanoke River in Montgomery County, Virginia.

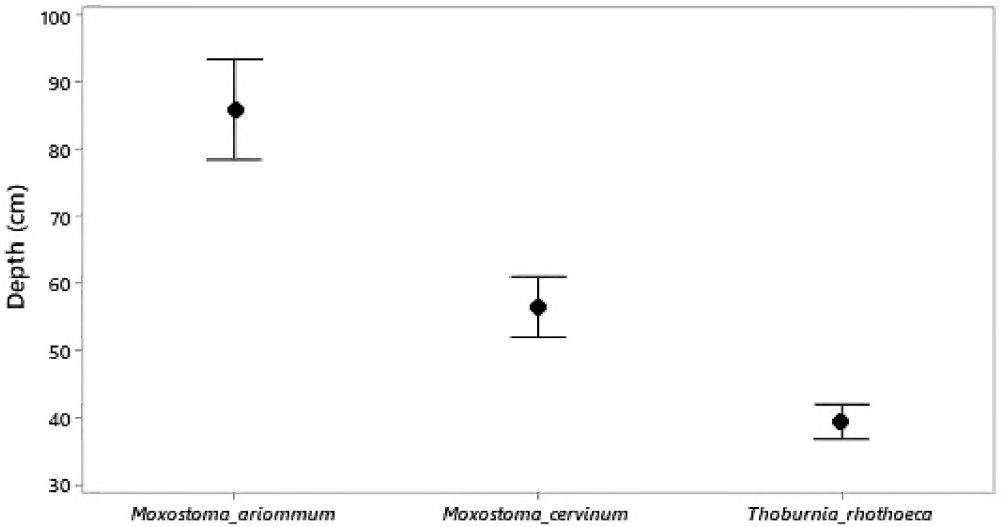

FIGURE 3: Plot of 95% confidence intervals of mean depth of water occupied for *Moxostoma ariommum* (n = 30), *M. cervinum* (n = 35), and *Thoburnia rhothoeca* (n = 36) in the South Fork Roanoke River in Montgomery County, Virginia.

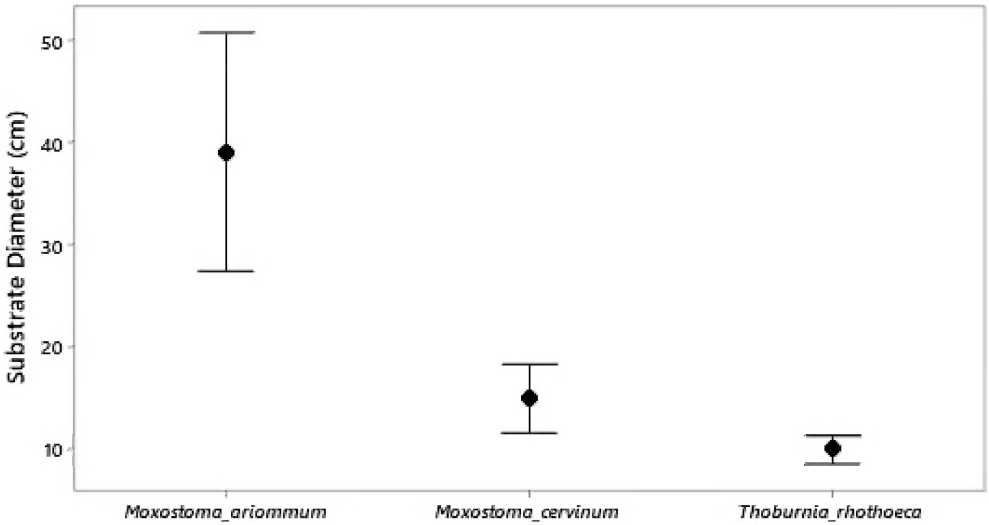

FIGURE 4: Plot of 95% confidence intervals of mean substrate diameter below *Moxostoma ariommum* (n = 30), *M. cervinum* (n = 35), and *Thoburnia rhothoeca* (n = 36) in the South Fork Roanoke River in Montgomery County, Virginia.

Mean fineness ratios were not equal among species ($P < 0.001$; mean for *Moxostoma ariommum* = 5.36, SE = 0.13; mean for *Moxostoma cervinum* = 5.04, SE = 0.08; mean for *Thoburnia rhothoeca* = 4.63, SE = 0.04). In the multivariate analysis of body shape, principal component two loaded heavily for eye width (-0.61), body width at pelvic fin origin (0.33), body width at anal fin origin (0.32), and body depth at dorsal fin origin (0.30). Principal component three loaded heavily for dorsal fin height (0.61) and caudal peduncle length (-0.47). The scatterplot of PC 2 and PC 3 reveal that *Moxostoma ariommum* has no overlap with *T. rhothoeca* or *M. cervinum* on PC 2 with the former having lower PC 2 scores than the latter two. There is minimal overlap among the latter two species along PC 3 with *T. rhothoeca* having higher PC 3 scores than *M. cervinum* (Fig. 5).

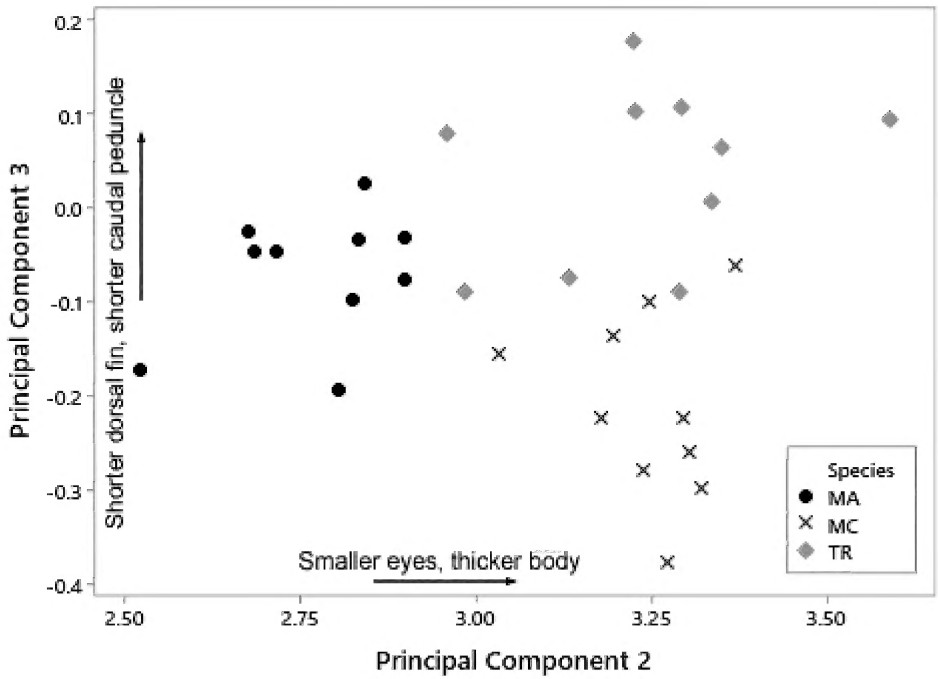

FIGURE 5: Scatterplot of Principal Component (PC) scores of 22 natural-log transformed body measurements of *Moxostoma ariommum* (MA), *M. cervinum* (MC), and *Thoburnia rhothoeca* (TR).

DISCUSSION

The habitat data suggest *T. rhothoeca, M. cervinum,* and *M. ariommum* occupy different microhabitats despite occupying the same stream reach within our study site. All three habitat variables appear to be important in habitat partitioning for these catostomids that share many ecological characteristics. *Thoburnia rhothoeca* occupies the fastest, shallowest water, with the smallest substrate. The mean value of 9.9 cm diameter and relatively small standard error of substrate for *T. rhothoeca* suggests this habitat is mostly cobble. This quantified microhabitat of fast, shallow water over cobble is consistent with the riffle habitat reported by Matthews (1990) for *T. rhothoeca*. *Moxostoma ariommum* occupies the slowest, deepest water over the largest substrate. This substrate is mostly bedrock consistent with habitat descriptions in Jenkins and Burkhead (1994) for *M. ariommum*. *Moxostoma cervinum* appears to inhabit habitat intermediate between that occupied by *T. rhothoeca* and *M. ariommum.* Collectively, these analyses can be interpreted as *T. rhothoeca* primarily occupying riffle habitat, *M. cervinum* occupying run habitat nearest the base of riffles, and *M. ariommum* occupying slower run habitat over bedrock substrate.

While the differences in habitat appear to be quite clear, associated morphological differences among these species are not necessarily as clear. With a fineness ratio of 4.63, *T. rhothoeca* appears very similar to the optimum for endurance swimming of 4.5 (Walker et al., 2013). The highest fineness ratio among species examined was in *M. ariommum* which inhabited the slowest water. Higher fineness ratios are common in burst-and-coast swimming patterns (Chung 2009). These burst-and-coast swimming patterns are often associated with maximizing sensory perception in fishes (Ashraf et al., 2020). With *M. ariommum* having a much larger eye than the other two species examined, the high fineness ratio and large eye may be associated with one another.

The functional significance of the larger eye of *M. ariommum* is not easily explained. Jenkins and Burkhead (1994) suggest it allows for greater detection of predators. The occupation of slower water by *M. ariommum* may make them more susceptible to predation especially over bedrock substrate with fewer crevices to hide from predators. Modest dietary differences exist among the study species (Jenkins and Burkhead, 1994; Tarasidis and Powers, 2014; Thompson et al., 2015); these differences may also be associated with contrasting eye sizes. *Moxostoma ariommum* appear to rely somewhat less heavily on chironomids and detritus than *T. rhothoeca* and *M. cervinum* and more heavily on larger aquatic insect nymphs. The large eye may be associated with increased reliance on sight feeding for those larger and more motile food items. Similarly, *Moxostoma lacerum* was hypothesized to use its large eye as an adaptation for sight feeding (Miller and Evans, 1965) on aquatic snails (Fink and Humphries, 2010) in slower run habitats (Jenkins and Burkhead, 1994) like those inhabited by *M. ariommum*. Mean eye size has been documented as inversely associated with depth in most fishes, but this relationship does not appear to be clearly linear. There is some evidence that a greater relative eye investment (i.e. size) is associated with dimly lit environments (Caves et al., 2017). As *M. ariommum* is a benthic fish in the deepest water of the species investigated in this study, it may inhabit more dimly lit waters than *M. cervinum* or *T. rhothoeca* possibly explaining the larger eyes of the former. Additionally, Caves et al. (2017) found increased acuity in more complex habitats. The larger substrate size beneath *M. ariommum* may represent a more complex habitat than inhabited by *M. cervinum* or *T. rhothoeca.*

The increased width and depth near the center of the body of *T. rhothoeca* and *M. cervinum* in comparison to *M. ariommum* may be associated with the contrasting current velocities inhabited by them. Langerhans et al. (2007) document a more fusiform body associated with increased water flow for Cyprinidae. The greater body depth and width near the middle of the body of *T. rhothoeca* and *M. cervinum* compared to *M. ariommum* suggest a similar phenomenon in Catostomidae with these more fusiform bodied species occupying the fastest water within our study site.

The smaller dorsal fin of *T. rhothoeca* compared to *M. cervinum* is consistent with the findings of Istead et al. (2015) who found dorsal fins in Centrarchidae reduced by faster flowing water. The smaller dorsal fin likely reduces drag helping to reduce exertion needed in the fast water inhabited by *T. rhothoeca.* However, this trend in a reduced dorsal fin did not extend to *M. ariommum* which inhabits the slowest water of all species examined in this study and has largely overlapping values for PC 3 scores with *T. rhothoeca.* The interaction between dorsal fin size and current velocity is likely complex as other authors investigating the influence of current velocity

on dorsal fin size found a direct relationship between them rather than an inverse relationship (Paez et al., 2008; Leavy and Bonner, 2009).

Variation in caudal peduncle length with variation in water flow has been documented with different authors reporting contrasting relationships in different species as Foster et al. (2015) reported a shortened caudal peduncle in response to faster water in *Goodea atripinnis,* Blackfin Goodea (Goodeidae) but the opposite in *Chirostoma jordani,* Mesa Silverside (Atherinopsidae). Istead et al. (2015) reported longer caudal peduncles for three species of Centrarchidae reared in faster waters. However, all of these species have very different gross morphologies than our study species and likely have vastly different overall hydrodynamics than the Catostomidae investigated in this study. Additionally, caudal peduncle length loaded heavily on PC 3 largely segregating *T. rhothoeca* and *M. cervinum.* As these two species occupy much more similar habitats than that of *M. ariommum,* we should expect differences among them to be more nuanced. This leaves the generally inverse relationship between caudal peduncle length and current velocity occupied difficult to clearly explain with any certainty.

As suggested in Domenici (2003) and Langerhans et al. (2007), the interactions between morphology and habitat are complex with selection working on individuals within the context of behavior and physiological characteristics where different taxonomic groups are likely to display different trends. Therefore, it is not surprising that our data show a mosaic pattern of relationships between divergent morphologies, habitats, and diets of these syntopic catostomid species. Collectively, these differences likely contribute to their resource partitioning and the maintenance of the diverse assemblage of catostomids in the Roanoke River drainage consistent with the summary of other taxa provided by Ross (1986). Additionally, increased sample sizes may lead to different findings.

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The Effect of Storm Events on Diet of Adult Mummichogs (*Fundulus heteroclitus*)

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ABSTRACT

More frequent storms due to climate change may impact estuarine species such as the mummichog (*Fundulus hetereoclitus*), an ecologically important salt marsh fish. This study investigated the effect of storm events and month on consumption of terrestrial insects by mummichogs in Hoffler Creek, Portsmouth, VA, as well as the effect of storms on consumption of major categories of benthic prey. Samples were taken monthly in the summers of 2017 and 2019. Additional paired samples were taken in June and July 2019, with the first collection during dry weather and the second during a subsequent storm. Month had a significant effect on the proportion of terrestrial insect prey in the diet in both years; consumption was highest in August, particularly in 2017 when the sample coincided with a storm event. However, storms increased consumption of terrestrial insects in only one of four paired dry weather-storm samples in 2019, indicating that temporal variation in insect abundance has a larger effect than increased availability that might occur when storms knock insects into the water. Storms had a significant effect on the proportion of different benthic prey in the diet in paired samples from 2019, but these effects were not consistent across months, sites, or in whether storms increased or decreased consumption. These patterns may be driven by mummichogs taking advantage of small-scale temporal or spatial variation in benthic prey. The ability to utilize locally abundant resources, including terrestrial insects, may help minimize the negative impacts of climate change on mummichogs.

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INTRODUCTION

Rising global temperatures are expected to cause an increase in intensity and frequency of storm events (Bender et al., 2010) that can have notable effects on the water chemistry and physical structure of aquatic habitats. Estuaries, in particular, can be impacted by drastic, but temporary, fluctuations in temperature (Wenner et al., 2004), salinity (Richmond and Woodin, 1996), and dissolved oxygen (Stevens et al., 2006) as a result of storms. Such changes in physical conditions affect species distribution, survival, and recruitment rates and may be especially pronounced in shallow estuarine habitats (Godley and Brodie, 2007; Rountree and Able, 2007; Muhling et al., 2018).

Salt marshes, in particular, are strongly influenced by both atmospheric conditions and overland flow (Hackney et al., 1976; Rountree and Able, 2007), leading to large changes in physical conditions during storms. Changes in temperature and salinity can have direct consequences on the survival and performance of resident salt marsh fish species (e.g., Collar et al., 2020), as well as affecting the abundance and availability of their prey. The mummichog (*Fundulus heteroclitus)* is an abundant resident of salt marshes along the east coast of the USA (Kneib, 1986). Although young individuals can remain in shallow pools on the marsh surface throughout the tidal cycle (Galleher et al., 2009), adult mummichogs in most systems move into the intertidal marsh with the flooding tide before returning to the subtidal creek at low tide (Teo and Able, 2003). Occupying the intertidal marsh provides mummichogs with refuge from predation (Banikas and Thompson, 2012) and an abundance of prey due to high levels of primary production (Kreeger and Newell, 2000) that support marsh invertebrate populations (Deegan et al., 2000). Mummichogs are opportunistic feeders that consume different prey items depending on body size and location within each subhabitat of the salt marsh (Thompson, 2015). Main prey items include terrestrial insects and small benthic invertebrates such as polychaetes, ostracods, and copepods (Kneib and Stiven, 1978; Allen et al., 1994; James-Pirri et al., 2001; Thompson, 2015).

Storm events could impact prey availability for mummichogs and other salt marsh fish species by causing immediate mortality of aquatic prey or by influencing the distribution of prey as they seek to avoid unfavorable water quality conditions. Studies on a variety of nearshore benthic communities find that those habitats become more homogenous with reductions in the abundance of invertebrate species as storms pass through (Blockley et al., 2007; Roberts et al., 2007; Corte et al., 2017). The availability of terrestrial insect prey, however, may be increased by storm events due to the higher likelihood of collisions between rain and insects, causing the insects to fall in the water and become more readily accessible for consumption by mummichogs. A previous study on mummichog diet in a Virginia tidal creek found that the level of terrestrial insect consumption was highest during a storm event in August (Thompson, 2015), and this pattern was even more pronounced in data collected in 2017 as part of an ongoing study on benthic prey selectivity of mummichogs (J. S. Thompson, unpublished data). Although these results suggest that storm events may increase consumption of terrestrial insects, samples in both studies were collected only once each month so the effects of rain, as opposed to monthly variation in insect abundance, could not be distinguished.

Little research has been done on how shifts in the abundance or availability of prey species during storm events directly impact resident salt marsh fish like the mummichog. The objective of our study was to observe the effects of storm events on the prey consumed by mummichogs found in intertidal salt marshes of a Virginia tidal creek, with a focus on the portion of the diet composed of terrestrial insect prey. We present monthly data on insect consumption collected in the summer of 2017 as part of a mummichog diet selectivity study, as well as data on insect and non-insect prey from paired dry weather-storm event samples collected in 2019 to better distinguish between the effects of storm events and seasonality. Mummichogs serve as an important prey species to many predatory estuarine fishes (Kneib, 1986; Nemerson and Able, 2003; Able et al., 2007), so understanding how storm events affect consumption of aquatic and terrestrial prey will help predict how this ecologically important fish may respond to climate change.

MATERIALS AND METHODS

Study Site

Mummichogs were collected from intertidal marshes at two sites along Hoffler Creek, a tributary of the James River, located in Portsmouth, VA, approximately 11 km from where the James River flows into the Chesapeake Bay. Hoffler Creek is brackish (12-28 ppt salinity) and has a mean tidal range of 0.84 m (Thompson, 2015). The intertidal habitat is composed of vegetated marsh (primarily *Spartina alterniflora*) and unvegetated mudflats. Mummichogs are abundant in the intertidal habitat at high tide but return to the subtidal creek at low tide (Thompson 2015). Hoffler Creek was selected for this study because it contains areas of fringing marsh with connection to the upland habitat that are typical of low to moderately impacted tidal creeks along the lower Chesapeake Bay, and the diet of mummichogs in Hoffler Creek (Thompson 2015) is comparable to other systems along the Atlantic coast (e.g., Kneib and Stiven, 1978; Allen et al., 1994; James-Pirri et al., 2001). This system also provided easy access to sampling sites both downstream, close to the mouth of the creek, and upstream, close to the creek's origin.

The downstream sampling site was approximately 485 m from the creek mouth and had an extensive marsh area along a wide and deep subtidal creek. Mummichogs were collected from the eastern edge of a large contiguous intertidal habitat with an area of 33,480 m^2 composed of approximately 73% vegetation and 27% intertidal mud flat or channel. The average intertidal width (measured from the subtidal creek to the upland habitat) was 106 m. The upstream site was located 1,790 m from the creek mouth. It had intermittent patches of fringing marsh along a narrow and shallow subtidal creek. Mummichogs were collected from the middle of a patch of marsh with an average intertidal width of 29 m and an area of 2,400 m^2, composed of approximately 82% vegetation and 18% intertidal mud flat.

Mummichog Collection

Mummichogs ≥40 mm total length were collected in unbaited, cylindrical minnow traps (22.9 cm diameter, 44.5 cm long, 6.4-mm mesh) following the protocol of Thompson (2015). In

brief, traps were set in areas of intertidal marsh vegetation within two hours of a daytime high tide to ensure diet items included in the study were consumed while fish occupied intertidal habitats. To minimize digestion of prey item, fish were removed from traps set for no more than 15 minutes. A maximum of 30 fish from each site on each sampling date was retained for inclusion in the study. After fish were euthanized in an MS-222 solution, a slit was made in the body cavity to allow for perfusion of the gut, and fish were placed in 10% buffered formalin for preservation. When more than 30 fish were captured, fish of different sizes were chosen in approximate proportion to their occurrence in the sample to account for size-specific differences in the diet (Thompson 2015), and the surplus fish were released at their site of collection. Mean total length of fish retained for diet analysis was similar across samples (Table 1).

TABLE 1. Sample size (number of fish and number of diet items identified) and total length (mm; mean ± s.d.) of mummichogs collected from downstream and upstream sites along Hoffler Creek, Portsmouth, VA, and included in analysis of the effects of month and storm events on diet. Single monthly samples were taken in May-August, 2017, and in August, 2019; paired dry weather and storm samples were taken in June and July, 2019.

	Downstream site			Upstream site		
	Number of fish	Number of diet items	Total length	Number of fish	Number of diet items	Total length
May, 2017 (dry weather)	22	397	64 ± 12	25	445	65 ± 13
June, 2017 (dry weather)	25	282	65 ± 13	25	834	60 ± 12
July, 2017 (dry weather)	25	236	65 ± 14	25	318	63 ± 13
August, 2017 (storm)	25	234	58 ± 12	25	311	54 ± 10
June, 2019 (dry weather)	25	78	65 ± 10	25	385	56 ± 5
June, 2019 (storm)	25	555	59 ± 12	19	184	57 ± 6
July, 2019 (dry weather)	25	177	63 ± 8	25	316	59 ± 7
July, 2019 (storm)	25	122	67 ± 12	25	438	60 ± 7
August, 2019 (dry weather)	25	126	60 ± 8	24	206	55 ± 7

To determine the effect of storm events on mummichog diet, prey items consumed during periods of dry weather were compared with those items consumed during storms. Dry weather samples were collected in periods without precipitation and at least 48 hours after any previous rainfall. The original study objective was to determine whether storm events make terrestrial insects more available to mummichogs by washing insects into the marsh from the upland habitat or knocking insects into the water from marsh grasses. In the absence of any established protocol for this type of study, 48 hours (four full tidal cycles) were assumed to be sufficient for any terrestrial insects washed into the marsh in a previous storm to be carried out of the habitat by tidal action and no longer be available for mummichog consumption. Storm samples were collected during precipitation events with at least 30 minutes of steady rain prior to minnow traps being set in the marsh. Thirty minutes of rain were assumed to be sufficient to increase mummichog access to terrestrial insects washed into the marsh.

In 2017, both upstream and downstream samples were taken on the same day in monthly collections from May through August. Samples from the first three months met the criteria for a dry sample, whereas the sample collected in August met the criteria for a storm sample.

In 2019, pairs of dry and storm samples were taken at both the upstream and downstream sites in June and again in July. Dry samples were taken no more than 3-5 days before the paired storm sample to ensure that temporal differences in prey availability independent of the occurrence of storms would be minimized. An additional dry sample was taken in August for comparison with dry samples from earlier months; no storms occurred during daytime high tides in this month, preventing collection of a corresponding storm sample.

Diet Analysis

In the lab, prey items were removed from the anterior portion of the gut (Sections I and II; Babkin and Bowie, 1928) to ensure that only the most recently consumed items were analyzed (Thompson, 2015). Identifiable food items were tallied for each fish using broad prey categories to allow for comparisons between dry and storm samples and across months. These categories included terrestrial insects, aquatic larval insects, polychaetes, copepods, ostracods, tanaids, and nematodes. Empty stomachs were excluded from the analysis. Twenty-five individuals were processed from each site and date (randomly selected from the 30 fish collected), except in cases where the number of fish collected was too low or the number of empty stomachs was too great to attain this sample size (Table 1).

Statistical Analysis

Mummichog diet was analyzed based on (1) the frequency of occurrence of terrestrial insect prey, defined as the proportion of fish in a sample with at least one terrestrial insect in the gut, and (2) the numerical proportion of terrestrial insect prey relative to non-insect prey for all fish in a given sample. The effect of month (May-August) on each metric was assessed in 2017 using 2×4 Chi-square tests for each sampling site. In 2019, 2×3 Chi-square tests were used to assess the effect of month (June-August) on the numerical proportion of terrestrial insect prey in dry samples. A small number of mummichogs consumed insects in 2019 so the Freeman-Halton extension of the Fisher's exact test for a 2×3 contingency table (Freeman and Halton, 1951) was used to assess the effect of month on frequency of occurrence in dry samples.

The effect of storm events on frequency of occurrence and numerical proportion of terrestrial insect prey was assessed for paired dry weather-storm samples (collected at each sampling site in June and July of 2019, as described above) using one-tailed Fisher's exact tests (Fisher, 1925). This test was chosen to accommodate the small number of terrestrial insects in the diet in 2019, and a one-tailed test was chosen based on the hypothesis that storms would increase insect consumption due to insects being knocked into the water by the rain.

Substantial variation was observed between paired dry weather and storm samples collected in June and July, 2019, for some categories of benthic prey. Therefore, post hoc analyses were conducted to test for significant effects of storms on the proportion of major benthic prey items, defined as accounting for ≥5% of total identifiable prey items on at least one sample date at one site. A two-tailed Fisher's exact test was used to compare the number of items in the given prey category versus items in the other major categories in paired dry weather and storm samples for each sampling site. The Bonferroni correction was used to determine the significance of each individual comparison in order to hold $\alpha=0.05$ across all 24 of the resulting post hoc tests.

RESULTS

Consumption of terrestrial insects was low to moderate in May through July of 2017 but increased in August at both sites, with the most substantial increase observed at the upstream site (Table 2). Month had a significant effect on the numerical proportion of terrestrial insect prey in the diet at both the downstream (X^2=34.02 with df=3, p<0.001) and upstream (X^2=967.97 with df=3, p<0.001) sites. At least 40% of fish in each sample from 2017 had at least one terrestrial insect in the gut, but this value increased to 96% in August at the upstream site (Table 2). Month had a significant effect on the frequency of occurrence of terrestrial insects in the diet at the upstream site (X^2=17.98 with df=3, p<0.001).

TABLE 2. Consumption of terrestrial insects by mummichogs at downstream and upstream sites along Hoffler Creek, Portsmouth, VA, in May through August of 2017. Samples from May through July were collected during dry weather, whereas the August sample was taken during a storm event. Values are the numerical percentage of total diet items that were terrestrial insects and, in brackets, the frequency of occurrence (the percentage of fish with at least one terrestrial insect in the gut). Sample sizes and mean fish lengths are provided in Table 1.

	May	June	July	August
Downstream site	4.5 [50]	8.2 [40]	5.5 [40]	17.1 [64]
Upstream site	6.1 [60]	3.1 [56]	10.3 [40]	77.8 [96]

Consumption of terrestrial insects by mummichogs was low throughout 2019 (Table 3). Although storm events generally increased the proportion of terrestrial insect prey in the diet (Table 3), this increase was only significant at the upstream site in June (one-tailed Fisher's exact test p=0.04). The frequency of occurrence of terrestrial insects in the diet was two to three times higher during storm events than in dry weather samples (Table 3), but this increase was not significant in paired samples from June or July at either site.

Comparison of dry weather samples from 2019 showed an increase in the numerical proportion of terrestrial insect prey in the diet in August at both sites (Table 3), but month had a significant effect on these proportions only at the upstream site (X^2=16.46 with df=2, p<0.001). The frequency of occurrence of terrestrial insects in the diet was also highest in August (Table 3), and again, monthly differences were significant only at the upstream site (Fisher-Freeman-Halton 2x3 exact test p=0.018).

TABLE 3. Consumption of terrestrial insects by mummichogs at downstream and upstream sites along Hoffler Creek, Portsmouth, VA, for paired dry weather and storm samples in June and July, as well as a dry weather sample in August, of 2019. Values are the numerical percentage of total diet items that were terrestrial insects and, in brackets, the frequency of occurrence (the percentage of fish with at least one terrestrial insect in the gut). Sample sizes and mean fish lengths are provided in Table 1.

	June		July		August
	Dry	Storm	Dry	Storm	Dry
Downstream site	5.1 [16]	2.3 [36]	3.4 [12]	6.6 [28]	8.7 [32]
Upstream site	0.5 [8]	2.7 [21]	1.3 [12]	1.8 [24]	5.3 [38]

Significant differences in the proportion of non-insect diet items consumed by mummichogs in paired dry weather and storm samples from June and July, 2019, were detected for all major prey categories except nematodes (Table 4). However, these differences were not consistent between sites or months, and for some prey categories, including copepods and ostracods at the upstream site, storm events caused a significant decrease in consumption of that item in one month and a significant increase in consumption in the other month (Table 4).

TABLE 4. Consumption of benthic prey by mummichogs at downstream and upstream sites along Hoffler Creek, Portsmouth, VA, for paired dry weather and storm samples in June and July of 2019. Values are the percentage of total diet items in that prey category and are shown only for major prey categories that accounted for ≥5% of diet items in at least one sample. Paired samples in bold are significantly different between dry weather and storm samples for that site in that month based on two-tailed Fisher's exact tests with a Bonferroni correction to ensure α=0.05 across all tests. Sample sizes and mean fish lengths are provided in Table 1.

	Downstream site				Upstream site			
	June		July		June		July	
	Dry	Storm	Dry	Storm	Dry	Storm	Dry	Storm
Polychaetes	**48.7**	**6.8**	48.6	33.6	9.6	17.4	15.8	10.3
Aquatic larval insects	2.6	9.5	9.6	10.7	**13.0**	**36.4**	9.2	6.4
Copepods	10.3	10.3	8.5	20.5	**6.8**	**35.9**	**43.0**	**3.0**
Ostracods	**25.6**	**3.4**	29.4	22.1	**68.1**	**5.4**	**29.4**	**78.3**
Tanaids	**1.3**	**66.1**	0.6	0.8	0.5	1.6	1.3	0
Nematodes	6.4	1.4	0	5.7	1.6	0.5	0	0.2

DISCUSSION

This study found consistent seasonal variation in the consumption of terrestrial insect prey by mummichogs (Tables 2 and 3), with the highest consumption observed in August, particularly in 2017. However, only one of the four paired samples collected in 2019 showed a significant increase in insect during storm events (Table 3), revealing that storms had a minor effect on the consumption of terrestrial insects by mummichogs. The main groups of terrestrial insects consumed in 2017 and 2019 were *Prokelisia* spp. (marsh-dependent planthoppers) and a variety of Diptera (flies and midges) that would be present in the marsh and at the marsh edge. Planthopper abundance can increase rapidly as a result of increasing host plant biomass and quality (Denno and Roderick, 1990; Gratton and Denno, 2003). In *Spartina alterniflora*-dominated marshes, this increase in plant biomass would be expected over the summer growing season (Dame and Kenny, 1986), potentially leading to increased planthopper abundance by August. Dipteran populations tend to increase with higher temperatures and precipitation rates (Alto and Juliano, 2001), which may contribute to the observed higher rates of consumption by mummichogs in August of both years.

The effect of precipitation on insect populations may also help explain the overall increase in insect consumption in 2017 compared to 2019, given that higher rainfall was recorded in Portsmouth, VA, over the summer in 2017 (68.7 cm from May to August) versus 2019 (51.1 cm over the same time period) (NOAA's National Centers for Environmental Information

Climate Data Online, https://www.ncdc.noaa.gov/cdo-web/). The highest levels of terrestrial insect consumption by mummichogs may, therefore, occur with a combination of high seasonal abundance, as in August in both years of this study, and high availability, as might occur during some storm events or after prolonged seasonal precipitation. This pattern is most evident in the very high insect consumption observed during the storm event in August 2017.

Both our upstream and downstream sampling sites had areas of dense marsh vegetation that would support populations of marsh-dependent insects, along with an unimpeded connection between marsh and upland habitats that may increase access to terrestrial insects at this ecotone. Except for August 2017, the consumption of terrestrial insects in a given month was similar at both sites, which was expected since each site has similar compositions of mud flat and vegetation. In addition to the *Prokelisia* spp. and Diptera mentioned above, storm samples collected in 2019 had a notable increase in the quantity of Formicidae (ants) consumed, particularly at the upstream site, and the increase in ant consumption led to the significant difference in the proportion of terrestrial insects consumed between dry weather and storm samples in June at that site. Ants can be abundant along the upland edge of marshes (Brandt et al., 2010), and storm events may lead to higher water levels that allow mummichogs to more easily access this edge habitat and may also increase the rate at which ants are washed into the marsh itself. This suggests that insect consumption overall depends on having both sufficient areas of marsh vegetation and access to marsh-upland edge and that increases in terrestrial insect availability with storms may be particularly dependent on upland access.

Substantial and significant differences in the consumption of most major non-insect prey categories were seen in at least one paired dry weather-storm sample in 2019 (Table 4). However, these differences were not consistent across sites, months, or the direction of change between dry weather and storm samples. Two possible explanations for this variation are that (1) the abundance of benthic invertebrate prey species varies over small spatial and temporal scales, independent of the influence of storms, and mummichogs then consume those locally abundant resources at higher rates, or (2) prey species respond to storms but variation in the most abundant species or life stage across spatial and temporal scales results in inconsistent patterns when comparing dry weather and storm samples. Substantial spatial variation in the abundance of major benthic invertebrate taxa has been reported over the scale of meters in a variety of soft-bottom nearshore and intertidal habitats (Morrisey et al., 1992; Paiva, 2001; Whaley and Minello, 2002; Ysebaert and Herman, 2002), and temporal variation is seen in many benthic invertebrate populations at time scales ranging from days to weeks (Morrisey et al., 1992; Whaley and Minello, 2002). Morrisey et al. (1992) also identified substantial interaction between patterns of temporal and spatial variation, such that most sampled taxa exhibited different temporal patterns even across plots at the same location; in the context of this study, that would suggest that prey species at the upstream and downstream sites may display different temporal patterns of abundance and lead to inconsistent patterns in consumption by mummichogs across even the short number of days between dry weather and storm samples.

Although the effect of storm events on benthic communities is not well studied, reductions in the immediate abundance of some taxa, most notably crustaceans, have been detected (Blockley et al., 2007; Roberts et al., 2007; Corte et al., 2017). Of the crustaceans found

in the mummichog diet in this study, consumption of ostracods declined significantly during storms in two paired samples and consumption of copepods declined in one paired sample. However, consumption of ostracods and copepods each increased significantly during storms in one additional paired sample, as did consumption of tanaids. These inconsistencies may reflect the fact that the effects of storms on abundance of some species would be layered over small-scale temporal and spatial variation in the composition of the benthic community (Morrisey et al., 1992), and different species even within the same taxonomic category may respond differently to storms, as observed for polychaetes by Corte et al. (2017). In addition, it is important to note that the means by which abundances of invertebrates in the benthos are reduced can impact their susceptibility to predators. If storms physically disrupt the substrate and scour invertebrates into the water column (Corte et al., 2017) or lead to increased movement by mobile individuals seeking to avoid adverse water quality conditions (Blockley et al., 2007), these individuals may become more available to mummichogs feeding in the habitat even as their abundance in the substrate declines.

The criteria used to designate dry weather and storm samples in this study were based on the primary objective of assessing the effect of storms on mummichogs' consumption of terrestrial insects. Specifically, it was expected that the availability of terrestrial insects responded fairly rapidly to changing precipitation patterns if the dominant mode of increased availability was insects being washed into the marsh or being knocked off of marsh grasses or overhanging vegetation during rain events. The time scale over which soft sediment benthic communities may be impacted by storms, however, is unknown, which raises the possibility that 48 hours without rain (the definition of dry weather used here) may be insufficient to negate latent effects of earlier storms. Similarly, a minimum of 30 minutes of steady rain (the definition of a storm event used here) may be insufficient for changes in the benthic community to occur. The fact that there were observed differences in mummichog diet in some paired dry weather-storm samples suggests that this sampling design captured differences in benthic prey availability based on precipitation patterns, but further research on the rate of recovery of intertidal, soft sediment benthic communities after storms will be important to fully predict how the diet of salt marsh fishes will respond to storm events.

The small number of storm events sampled in this study also limits the ability to discern how specific characteristics of storms may affect benthic invertebrates and terrestrial insects, as well as resulting patterns in fish diet. The amount of rainfall in each storm event included in this study was comparable (1.2-1.6 cm in 24 hours), but the amount of rainfall in the 48 hours prior to sampling was higher in August 2017 (4.6 cm) and June 2019 (3.6 cm) than in July 2019 (no rainfall; NOAA's National Centers for Environmental Information Climate Data Online, https://www.ncdc.noaa.gov/cdo-web/). Rainfall over the days leading up to storm samples would result in higher water levels in the marsh, increasing access to upland habitat and insects such as ants, which were observed in high numbers in the upstream June 2019 sample. Storm sampling is necessarily opportunistic, and unfortunately, there were a limited number of opportunities to collect paired samples in 2019. Future sampling across storms with a broader range of characteristics may help to determine which components of these storms are most important for

increasing fish access to terrestrial insects and for modifying benthic communities in ways that affect fish diet.

Overall, these findings suggest that the consumption of terrestrial insects by mummichogs depends mainly on seasonal abundance and, to a lesser degree, increased availability due to storms. Virginia is in the middle latitudes expected to experience more pulsed precipitation events due to climate change (Trenberth, 2011), and average precipitation in the Tidewater region, particularly in autumn months, has increased in the last two decades compared to historical values (Hoffman et al., 2019). Climate change is further predicted to increase insect population growth in species living in middle to high latitudes due to higher fitness brought on by warmer temperatures closer to the species' optimum range (Deutsch et al., 2008). The stronger storms created by these warmer temperatures can have varying effects on insect species based on location (Gandhi et al., 2007), although more research is necessary. The ability of mummichogs to take advantage of terrestrial insect prey, in addition to locally and temporally available benthic prey, may allow mummichogs to maintain a more robust diet and help to buffer negative impacts of climate change on this ecologically important species. However, the utilization of terrestrial insect prey by mummichogs is dependent upon preservation of sufficient marsh area, which can be destroyed or severely impacted by strong storms (Scavia et al., 2002), and unimpeded marsh-upland connections, which can be disrupted by shoreline hardening put in place to protect upland development from sea level rise (Gittman et al., 2015). Shoreline hardening and coastal development can also negatively impact the benthic macroinvertebrates (Bilkovic et al., 2006) that mummichogs depend on for the majority of their diet. Thus, preserving salt marsh habitat will be essential to support stable mummichog populations that serve as an important link in the estuarine food webs of Virginia and the broader Atlantic coast (Kneib, 1986).

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Identification of *Planktothrix* (Cyanobacteria) Blooms and Effects on the Aquatic Macroinvertebrate Community in the Non-Tidal Potomac River, USA

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ABSTRACT

Using transverse cross-sectional transects, a survey of 31 km of the non-tidal Potomac River was conducted from White's Ferry, Virginia, to Brunswick, Maryland, USA, between June and September in 2013 through 2015 to assess a recurring benthic cyanobacteria bloom. Abundant benthic cyanobacteria blooms were detected during the 2014 and 2015 sampling seasons and the primary taxon was identified morphologically and molecularly as *Planktothrix* cf. *isothrix*. When present, *P.* cf. *isothrix* blooms were concentrated from river center to the Maryland shoreline. This pattern was correlated with significantly greater benthic chlorophyll-*a* and phycocyanin concentrations. In an apparent response to the *P.* cf. *isothrix* blooms in the study site, aquatic macroinvertebrate community assemblages were significantly different between areas with extensive benthic cyanobacterial growth compared to areas without cyanobacterial growth. Within the *P.* cf. *isothrix* mats, the percentage of pollution sensitive taxa was lower, and the percentage of pollution tolerant taxa was greater. These data suggest that *P.* cf. *isothrix* can act as an ecosystem disruptor through direct impacts to the aquatic

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macroinvertebrate abundance and community structure within this section of the freshwater, non-tidal Potomac River.

Keywords: aquatic insects, ecosystem disruption, harmful algal bloom, Hilsenhoff Biotic Index, water quality

INTRODUCTION

The role of aquatic macroinvertebrates in the ecosystem is well documented. In general, aquatic macroinvertebrates play crucial roles in riverine nutrient and carbon cycling as they are major contributors both as consumers and as prey (Wallace and Webster, 1996; Malmqvist, 2002) and are a valued source of nutrition in recreational fisheries (Losey and Vaughan, 2006). Aquatic macroinvertebrate community composition can be influenced by multiple factors such as physical habitat, flow, temperature, geomorphology, and land use (Malmqvist, 2002; Ashton et al., 2014). Because of these predictable influences, aquatic macroinvertebrates have proven to be reliable and comprehensive bioindicators of water quality due to individual tolerance and sensitivity thresholds (Mandaville, 2002).

Aquatic macroinvertebrate communities have been monitored annually within the non-tidal Potomac River watershed since 1976 by the Maryland Department of Natural Resources (MD DNR) (Friedman, 2009; Saville et al., 2014). Analyses of aquatic macroinvertebrate communities at sites within the non-tidal Potomac River basin sampled between 2000 through 2014 categorize the communities as "good" using both the Hilsenhoff Biotic Index (HBI) and the Index of Biotic Integrity (IBI) (Friedman, 2009; Saville et al., 2014). However, these broad surveys have not looked at the discrete relationships that may exist between the aquatic macroinvertebrate community and microhabitats.

While nutrient loading of freshwater systems is a worldwide problem with implications for both water quality and ecological integrity, reductions in both nitrogen (N) and phosphorous (P) have been well-documented for the freshwater Potomac River over the past three decades (Langland et al., 2012; Moyer et al., 2017). However, current nutrient levels can still promote the nuisance growth of aquatic plants (including cyanobacteria, algae, and submerged vegetation), especially in areas of the freshwater Potomac River where the shallow depth profile permits sunlight to contact the substrate (Moyer et al., 2017). Recently, in the lower non-tidal Potomac River algal blooms of a filamentous cyanobacteria species that cycles from the benthic to pelagic environment have resulted in unsightly and odorous mats that discourage recreational use.

On the United States west coast, benthic cyanobacteria blooms reported in several rivers have been responsible for domestic animal mortalities (Puschner et al., 2008; Bouma-Gregson et al., 2017) and have contributed to biotoxin loads in local and downstream waters (Fetscher et al., 2015). There is also increasing evidence that many cyanobacteria blooms negatively impact aquatic life by reducing community diversity and survival rates, resulting in increased impairment designations (Aboal et al., 2000; Suren et al., 2003a; Oberholster et al., 2008; Tourville-Poirier et al., 2010; Anderson et al., 2018). Globally, many cyanobacteria species cause concerns for human

health when they occur in high concentrations and/or produce toxic or taste and odor compounds (Paerl and Otten, 2013).

Proper identification of cyanobacteria bloom species is required to understand the risk these blooms pose to human and environmental health as different species can produce different toxins under different environmental regimes (Harke et al., 2016; Bouma-Gregson et al., 2019). The identification of cyanobacteria has traditionally been based on morphological and ecological characteristics; however, as morphological characteristics can be variable depending on environmental conditions (Komárek and Anagnostidis, 2005), recent investigations have recommended the incorporation of genetic sequence analyses to confirm identifications (Dvořák et al., 2015; Gaget et al., 2015).

This is the first report of *Planktothrix* cf. *isothrix* blooms in the non-tidal, freshwater portion of the Potomac River. An investigation along a 31 km stretch of the lower non-tidal Potomac River was conducted over the course of three summers (2013-2015) to: 1) confirm the identification of this benthic to pelagic cyanobacteria species using morphological, ecological, and phylogenetic characteristics; 2) determine the spatial and temporal extent of the benthic stage of this cyanobacteria species within the study area; and 3) examine changes to the local aquatic macroinvertebrate community in the presence of this benthic cyanobacterium.

MATERIALS AND METHODS

Study Area

The non-tidal Potomac River has a watershed which drains roughly 29,900 km², encompassing Pennsylvania, Maryland, West Virginia, and Virginia. The Chesapeake and Ohio (C&O) Canal parallels the non-tidal portion of the Potomac River in its entirety, offering a forested and protected buffer along the Maryland border. Adjacent borders to the river in West Virginia and Virginia are privately or commercially maintained and offer much less riparian protection. While sediment and nutrient loadings have been improving compared to previous decades, eutrophication and sedimentation continue to be problematic in this system (Murphy et al., 2011; Langland et al., 2012; Moyer et al., 2017). The non-tidal Potomac River is Maryland's most popular non-tidal fishing destination (MD DNR, 2017) and is widely used for other recreational activities.

Field Study Design

To investigate the impact of benthic cyanobacteria blooms on the aquatic macroinvertebrate community three transverse cross-sectional transects were established along a 31 km stretch at three geographical locations in the lower section of the non-tidal Potomac River: White's Ferry, Point of Rocks, and Brunswick (Fig. 1; Appendix Table 1). Each transect contained three plots: one 20 m from each shoreline (Maryland [MD] and Virginia [VA]), and river center (RC). Each plot was sampled biweekly by boat from May through September (2013 – 2015) and the following water quality variables were recorded: water temperature (^{o} C), conductivity (μS cm^{-3}), dissolved oxygen (DO, mg L^{-1}), pH, and the concentrations (as relative fluorescence units, RFU) of the accessory pigments chlorophyll-*a* (chl-*a*) and phycocyanin (PC) using a pre-calibrated YSI

EXO 1 sonde (YSI Incorporated, Yellow Springs, OH, USA) positioned horizontally on the river bottom. To estimate percent coverage of the cyanobacteria mats, a 1.0 m² quadrat was tossed out from the sampling points along the transect (Appendix Table 1) and the percent of the quadrat covered by cyanobacteria was visually estimated following the method described by Steneck and Dethier (1994).

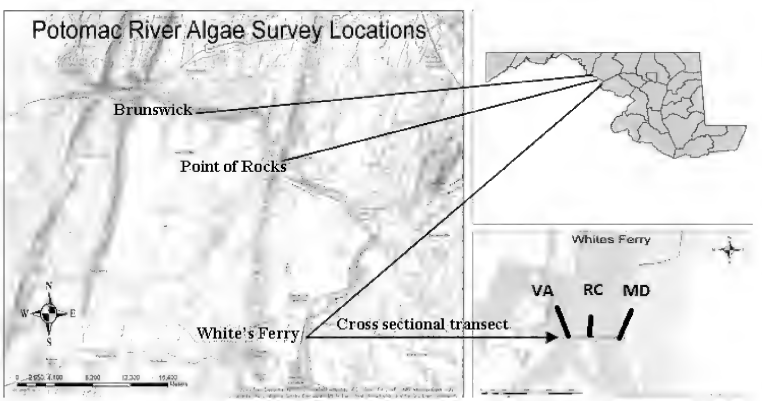

FIGURE 1. Survey locations in the lower non-tidal Potomac River, with an example of a cross-sectional transect used in the 2013-2015 survey.

Cyanobacteria Identification

Benthic algal mat samples collected in July 2014 from the Brunswick, Point of Rocks, and White's Ferry areas of the Potomac River were examined using a Zeiss Axiovert 200 inverted light microscope (Carl Zeiss, Thornwood, NY, USA) to determine the genus identification of *Planktothrix*, a filamentous, non-heterocystous cyanobacteria. Wet mounts of live and formalin-preserved material were examined at 400x and 640x magnification using the morphometric characteristics outlined by Suda et al. (2002) and Komárek and Anagnostidis (2005). Photomicrographs were acquired using an Olympus DP73 digital camera (Olympus America, Center Valley, PA, USA) for comparison with other known *Planktothrix* species. Throughout the course of the study benthic algal mats were collected at each sampling site to confirm the presence of *Planktothrix* using microscopy.

A 10 mL subsample from the benthic algal mat collected from Brunswick, MD in July 2014 was centrifuged at 15,000g to form a pellet of cyanobacterial cells. Total DNA was extracted from the pelleted cells following the manufacturer protocol for TRIzol (Invitrogen, Carlsbad, CA, USA). Universal cyanobacteria primers CYA106F and CYA781R (Appendix Table 2; Nübel et al., 1997) were used to amplify the 16S region of the rRNA gene cluster of *Planktothrix*. End point polymerase chain reaction (PCR) settings for *Planktothrix* amplification were 35 cycles at 94°C for 60 s, annealing for 60 s at 60°C, followed by extension at 72°C for 1 min. The presence of a ~616 base pair (bp) PCR product was confirmed using a 1% agarose gel electrophoresis and visualized using ethidium bromide staining. The PCR products were purified and cloned into

chemically competent *E. coli* using the Topo TA cloning vector (Invitrogen). Plasmid DNA was purified using the GenElute Plasmid Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA). Plasmid DNA was sent to the University of Maryland Center for Environmental Science at the Institute of Marine and Environmental Technology (Baltimore, MD, USA) for Sanger sequencing. The resulting 16S rRNA sequence was deposited in the National Center for Biotechnology Information (NCBI) GenBank with Accession number MH299785.

To identify the species of Potomac River *Planktothrix*, the resulting 16S rRNA sequence was compared to other known *Planktothrix* sequences (Appendix Table 3) using nucleotide BLAST (Altschul et al., 1990) to search the NCBI nonredundant database. Species were compared by creating a multi-alignment using the 616 bp region present in the sequenced clone and available sequences in GenBank using Clustalw. The alignment, formatted with the Boxshade 3.21 -ExPASy portal (Corpet, 1988; Artimo et al., 2012), indicated there was not a 100% match between the Potomac River *Planktothrix* and other known *Plankothrix* species. Using the 16S rRNA sequences, a phylogenetic consensus tree was constructed based on maximum likelihood (ML), maximum parsimony (MP), and neighbor joining (NJ) methods in Mega 7 (Kumar et al., 2016) to determine the relationship between the *Planktothrix* species. *Nostoc punctiforme* was used as an outgroup when constructing the consensus tree. The ML analysis was performed based on the Jukes-Cantor model, using all sites, with 1,000 bootstrap resampling per Kumar et al. (2016). The MP tree was based on the subtree-pruning regrafting algorithm with a search level of 1, with 10 random additions of sequences and 1,000 bootstrap replicates (Nei and Kumar, 2000). The NJ tree was computed with the Jukes-Cantor model and 1,000 bootstrap replicates.

Toxin Assays

Planktothrix benthic mat and overlaying water column samples from Brunswick, MD (29 July 2015) and White's Ferry, MD (17 August 2015) were collected and frozen at -20˚C to use for the analysis of the toxin microcystin. The frozen samples were subjected to three freeze/thaw cycles before using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (#PN 520011, Eurofins Abraxis, Warminster, PA, USA) that tests for the ADDA moiety of microcystin. The ELISA toxin assays were performed according to the manufacturer's protocol.

Aquatic Macroinvertebrates

In 2013 and 2014 aquatic macroinvertebrate samples were collected once per month (June – September) at each survey point to identify possible relationships between macroinvertebrate populations and *Planktothrix* blooms. Aquatic macroinvertebrates were collected using three 30-second kicks at each sample plot (MD, VA, RC) according to the methodology described in MD DNR (2017). Briefly, sampling began at the downstream end of the plot and proceeded upstream. Using a 600-micron mesh D-net, three 30-second kicks were conducted by positioning the net and disturbing the bottom directly upstream of the net, using a kicking and stomping motion of the foot, dislodging the upper layer of cobble or gravel and scraping the underlying bed. The net contents were then placed into a 5-gallon bucket of water. Bucket contents were sieved through a three-sieve sorting system composed of a 13.2 mm, 1 mm, and 600 µm mesh sequence (U.S. Standard Sieves, W.S. Tyler, Inc., Mentor, OH, USA). Aquatic macroinvertebrates were handpicked in the field from each sieve and combined into vials containing 90% isopropyl alcohol

so that one sample was obtained for each sample plot (MD, VA, RC). The term macroinvertebrate for this study references those animals > 600 µm. The aquatic macroinvertebrates were identified at 100x using an Olympus 10x/22 dissecting microscope (Olympus America). Specimens were identified to the lowest taxon possible based on Merritt and Cummins (1984), Pennak (1978), Stewart and Stark (1988), and Wiggins (1977).

To investigate the impact of the benthic stage of this cyanobacterium on the aquatic macroinvertebrate community, we compared samples collected in areas with high cyanobacteria coverage to those devoid of cyanobacteria growth during the 2015 bloom season. Using the same aquatic macroinvertebrate collection procedures outlined above, during the cyanobacteria bloom peak in 2015 we collected samples within cyanobacteria mats and outside of cyanobacteria mats (n=6) along the Brunswick transect.

Field Data Analysis

The following calculations were used to summarize aquatic macroinvertebrate community data, per site along the transect each month:

1) Richness = number of species or taxa in the sample.

2) HBI = Hilsenhoff Biotic Index, formula as modified in Bode (1988).

Tolerance values were adapted from Maryland Biological Stream Survey (Southerland et al., 2005), assigning tolerance values to each species. The HBI formula calculates a rating value for the whole sample using the following:

$$HBI = \sum^{s} (n_t * T_t) / N$$

where s = taxa in the sample; N = total number of individuals in the sample, or sample size; n_t = number of specimens in each taxon; T_t = tolerance value for each specific taxon. The HBI values and interpretations are given in Appendix Table 4.

3) Diversity = the dispersion of the specimens among species in the sample. The analysis is derived using the Shannon-Wiener (US EPA, 1973) formula as follows for diversity of individual species:

$$H' = \sum pi\ (\ln\ (pi))$$

Where pi = relative abundance of each group of organisms; values above 3.00 indicate undisturbed waters while those less than 1.00 are severely degraded.

4) Equitability = results derived from Shannon-Wiener (H') are used as the numerator in the Shannon-Wiener based equitability formula to obtain a comparative analysis among the samples (species evenness). The equitability formula is as follows:

J = H' / H' max, where H' max = ln S (S = # of species)

These formulas compare the theoretical number of species associated with each diversity measure to the actual value. Equitability is measured on a scale of 0 to 1. The closer to 1, the closer the sample comes to the theoretical maximum. In general, the higher the equitability value, the healthier the population.

5) EPT Index Number = number of specimens in orders Ephemeroptera, Plecoptera, and Trichoptera in the sample. Reported as %EPT, these specific taxa are indicative of more pristine ecosystems, with larger numbers indicating healthier ecosystems (Lenat, 1988).

6) Functional Feeding Group Classification (FFG) = adapted from Maryland Biological Stream Survey (Southerland et al., 2005).

A principal component analysis (PCA) was performed on the water quality/accessory pigment data to help identify the years when cyanobacteria blooms occurred. The analysis generated axes of correlated habitat variables that represented the greatest spatiotemporal variation in water quality for this dataset. Following analysis of the Scree Plot, the first three principal component axes were retained for subsequent analysis because they represented the majority of variance in the dataset. Spatial and temporal differences in water quality were tested using a two-way analysis of variance (two-way ANOVA) of PCA scores (dependent variable) because cyanobacteria blooms differed in intensity across years and locations in the river. Location of sample (i.e., MD, RC, and VA) and year (2013, 2014, 2015) were independent variables.

Aquatic macroinvertebrate relative abundances were analyzed using ordination analyses to identify patterns of change in relation to *Planktothrix* blooms. Within-year changes in aquatic macroinvertebrates were examined with a non-metric multidimensional scaling analysis (NMS). This exploratory approach allowed for the analysis of the data and for testing the hypothesis that aquatic macroinvertebrate communities differed significantly in the presence of *Planktothrix* blooms. Prior to NMS analysis, aquatic macroinvertebrate abundances were transformed by the square-root to minimize variance. A general relativization by species, which is appropriate when using Sorensen (Bray-Curtis) distances to quantify site-specific dissimilarities in species abundances, was used. Ordination analyses were performed with PC-ORD for Windows (McCune and Mefford, 2018). Plots of NMS axes that fit the data well (i.e., had low stress) were examined to visualize changes in community structure throughout summer (June – September) and among sampling locations (MD, RC, and VA). To determine if community structure differed between years, which differed in bloom intensity, a perMANOVA was used to test for the effect of year. PerMANOVA is a non-parametric, multivariate analysis that computes sum of squares from distances among sample sites in a balanced sampling design (Anderson, 2001). The probability that an F-value from a randomized dataset was greater than or equal to that observed was computed from 10,000 permutations of randomizing the dataset.

Three sites were sampled for macroinvertebrates in 2015 where *Planktothrix* blooms occurred, and these data were compared with similar data from nearby areas where blooms did not occur. These data were used to qualitatively compare percentages of relative abundances of taxa between habitats. Additionally, multi-response permutation procedures (MRPP) were used to test whether communities differed between areas with blooms and those with no blooms. Similar to perMANOVA, MRPP is a nonparametric test that uses randomization to compare parameters from real datasets to those from null datasets that are expected from randomization. The MRPP was done with unweighted, non-relativized data and Bray-Curtis distances. Significant difference in community structure between habitats with and without *Planktothrix* blooms was determined with a test statistic (T) that represents the difference between the groups. The T-value, resulting from

observed and expected within group average Bray-Curtis distances, and the probability of obtaining that within-group distance from a randomized data set (p) were computed. Within-group agreement (A) is a measure of effect that is usually less than 0.1, with values of 0.3 considered high (McCune et al., 2002).

One-way analysis of variance (one-way ANOVA) was used to determine differences among individual water quality values and aquatic macroinvertebrate EPT and HBI results. Tukey-Kramer post-hoc tests were used to interpret results computed from one-way ANOVA.

A bivariate linear regression model was used to determine the relationship between water temperature and *Planktothrix* blooms, where PC values were the dependent variable. This model type was also used to illustrate the relationship between aquatic macroinvertebrate functional feeding groups (dependent variable) and PC values. Individual functional feeding group concentrations were log-transformed prior to analysis.

RESULTS

Cyanobacteria identification

The Potomac River bloom-forming cyanobacterium was identified as *Planktothrix* cf. *isothrix* based on morphology and life history. Diagnostic morphological characteristics of this cyanobacterium were straight, non-attenuating trichomes with rounded apical cells and slight constrictions at the crosswalls (Fig. 2a). Cells were shorter than wide or isodiametric with an average width of 7.2 (SD = 0.36) μm and length of 3.9 (SD = 0.49) μm (n = 40). Blooms initiated as dark green tufts attached to the benthos (Fig. 2b) before becoming free-floating in the water column, similar to the benthic to pelagic life history reported for *P. isothrix* in other locales (Komárek and Anagnostidis, 2005).

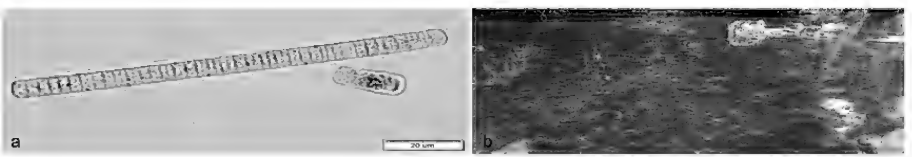

FIGURE 2. Light micrograph of *Planktothrix* cf. *isothrix* trichomes (a) and dark green tufts of *P.* cf. *isothrix* forming benthic mats in the Brunswick area of the Potomac River (b).

Additionally, a 616 bp region of 16S rRNA from the Potomac River *Planktothrix* was compared to known *Planktothrix* sequences. The BLAST sequence multi-alignment (not shown) indicated a 98% match between the Potomac River *Planktothrix* (MH299785) and *Planktothrix* sp. (strain UVFP1; AJ630646) from Spain (Camacho et al., 2005). A phylogenetic consensus tree generated using ML, MP and NJ analyses of 16S rRNA data (Fig. 3) shows the relationship between 18 known *Planktothrix* species and the Potomac River *Planktothrix*. Included in this

analysis was *Planktothrix agardhii* var. *isothrix* (AB045913; Suda et al., 2002). The Potomac River *Planktothrix* did not group with *P. agardhii* var. *isothrix* but formed a clade with *Planktothrix* sp. (strain UVFP1) from Spain (AJ630646; Camacho et al., 2005). Suda et al. (2002) synonymized their *P. agardhii* var. *isothrix* strains with *P. agardhii* based on morphological characteristics and a lack of genetic differentiation. Other *Planktothrix* species with benthic to pelagic life cycles, *P. clathrata*, *P. cryptovaginata*, and *P. tepida*, were not genetic matches with the Potomac River species. To our knowledge no genetically confirmed specimens of *P. isothrix* are available for comparison, hence the designation here as *P.* cf. *isothrix*.

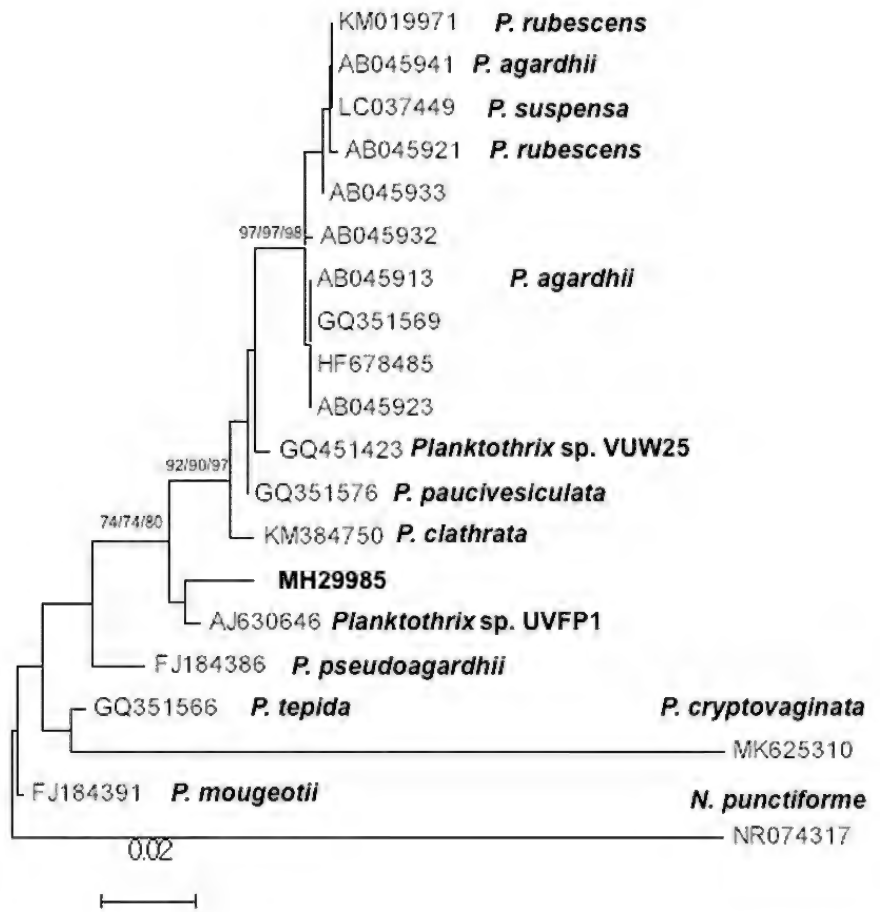

FIGURE 3. Maximum likelihood phylogeny of the 16S ribosomal RNA region for 19 *Planktothrix* sequences, representing 8 species groups and one out-group (*Nostoc punctiforme*). The numbers above the branches represent ML/NJ/MP bootstraps values (each branch represents 1000 replicates). Each sequence is identified by the GenBank Accession number. Sequence from this study is in bold.

Toxin analysis

The two *P.* cf. *isothrix* mat samples collected in 2015 tested positive for the presence of the hepatotoxin microcystin. Using ELISA, trace amounts of microcystin were detected (0.49 and 0.81 μg L^{-1}, expressed per 5 g wet weight). Whole water samples collected from the water column overlaying the *Planktothrix* mats were negative for microcystin.

Planktothrix cf. *isothrix* and Water Quality Variables

The 2013 dataset represented a year when nuisance levels of *P.* cf. *isothrix* did not occur versus 2014 and 2015 when *P.* cf. *isothrix* proliferated throughout the 31 km study area in the lower, non-tidal Potomac River. Benthic blooms of *P.* cf. *isothrix* were observed (within quadrats) 12 times at both Brunswick and Point of Rocks, and five times at White's Ferry in 2014 and 2015. Estimates of quadrat coverage data are presented in Table 1. It should be noted that low visibility on the Maryland shoreline prevented accurate observations of the benthos during most of July 2014 which likely underestimated coverages. These data revealed spatial and seasonal trends in pigment concentrations (chl-*a* and PC) and *P.* cf. *isothrix* distribution/coverages (Table 1). There was a relationship between PC pigment RFU and quadrat coverage estimates (Spearman's rank correlation, $r = 0.13$; $p = 0.04$), indicating that PC pigment concentration is a viable surrogate for estimating benthic cyanobacteria coverages.

TABLE 1. Mean spatial and temporal accessory pigment concentrations (chlorophyll-*a* and phycocyanin, measured as relative fluorescence units [RFU]) and quadrat coverage estimates for Maryland (MD), River Center (RC) and Virginia (VA) sites in the non-tidal Potomac River. Data was collected between June and September of 2013 through 2015 with observation and RFU values obtained from the river bottom.

Year	Shore-line	Sample size (N)	Non-detects (# occurrences)	Algae present (# occurrences)	Max quadrat (%)	Mean quadrat (%)	Mean phycocyanin	Mean chlorophyll-*a*
2015	MD	31	17	14	90	9.8	0.52	0.40
	RC	31	23	8	65	6	0.55	0.51
	VA	31	17	14	80	7.4	0.41	0.35
2014	MD	18	11	7	35	3.9	0.53	0.3
	RC	18	11	7	50	5.8	0.58	0.42
	VA	18	15	3	40	2.8	0.36	0.01
2013	MD	36	35	1	5	0.01	0.41	-0.13
	RC	36	34	2	5	0.03	0.42	-0.09
	VA	36	29	7	50	4	0.41	-0.12

Three principal component axes accounted for 75.2% variance in the water chemistry dataset. Of those, the first principal component (PC1) accounted for 32.5% of the variance in the original dataset and was highly correlated with chl-*a* and PC (Table 2). These data illustrated significant temporal and spatial differences in a gradient highly correlated with accessory pigments associated with algal blooms (Table 3). Differences existed temporally between years, where PC values were significantly higher in 2014 and 2015 than in 2013 (two-way ANOVA: $F = 33.6$, $p = <0.0001$) (Fig. 4). Significant spatial differences, across cross sectional transects (MD, VA, and RC), were also detected in those years when blooms occurred (one-way ANOVA: 2015: $P = 0.005$; 2014: $p = <0.0001$; 2013: $p = 0.3$) (Fig. 4). The chl-*a* and PC concentrations in 2014 and 2015 differed significantly (one-way ANOVA: $F = 30.1$, $p = <0.0001$; Tukey-Kramer Post-Hoc = 0.001) between the Maryland and Virginia shorelines (Fig. 5), with higher levels measured along the Maryland shoreline.

TABLE 2. Principal component analysis (PCA) axis loadings for water quality monitoring data collected in the summers of 2013 through 2015. Bold numbers represent the two main parameters for each axis.

Water Quality Parameter	PC1	PC2	PC3
Temperature °C	-0.1340	-0.1715	**0.7264**
Conductivity µS/cm	0.1670	0.0469	**0.6529**
Dissolved Oxygen mg/L	-0.2278	**0.7142**	0.0334
pH	-0.4330	**0.5242**	0.1105
Chlorophyll-*a*	**0.6080**	0.2657	0.1576
Phycocyanin	**0.5874**	0.3359	-0.0885

TABLE 3. The two-way ANOVA results for PC1 scores (Accessory Pigment Axis – Chlorophyll-a, Phycocyanin) for water quality monitoring data collected in the summers of 2013 through 2015.

	Sum of sqrs	df	Mean square	F	P
Temporal	104.06	2	52.03	36.6	< 0.0001
Spatial	27.34	2	13.67	9.6	< 0.0001
Interaction	12.10	4	3.03	2.1	0.08
Within	245.58	243	1.42		
Total	489.08	251			

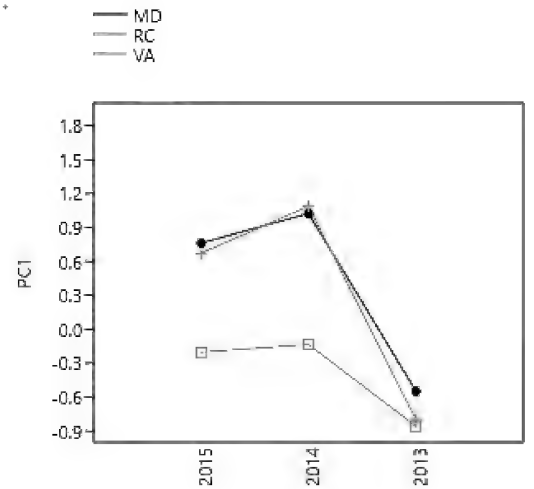

FIGURE 4. Plot of mean PC1 scores (chlorophyll-*a* and phycocyanin values) by year (2013, 2014, and 2015) and location (MD, RC, and VA). MD, RC, and VA signify Maryland, River Center, and Virginia sample locations, respectively.

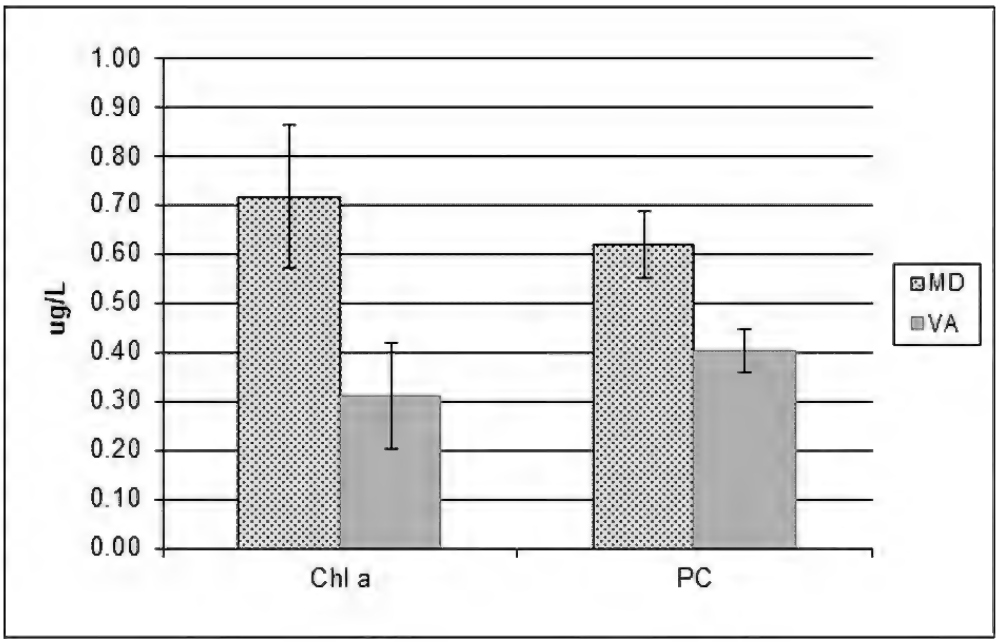

FIGURE 5. Annual mean accessory pigment values for *Planktothrix* monitoring at MD (n = 26) and VA (n = 26) Potomac River sampling stations. Error bars represent 95% confidence interval.

The second principal component (PC2) accounted for 23.2% of the variance in the original dataset. The PC2 was correlated with DO and pH (Table 2). The PC2 scores differed among years (two-way ANOVA: $F = 7.3$, $p = 0.001$) and between shorelines (two-way ANOVA: $F = 5.8$, $p = 0.004$), following similar trends to that observed in PC1.

The third principal component (PC3) accounted for 19.5% of the variance in the original dataset and was highly correlated with conductivity and temperature (Table 2). Similar to PC1, PC3 scores illustrated significant spatial (two-way ANOVA: $F = 4$, $p = 0.02$) differences. A difference in temperature and conductivity values was evident across transects. These values were significantly higher in 2014 and 2015 than 2013 (one-way ANOVA: $F = 6.1$, $p = <0.05$; Tukey-Kramer Post-Hoc: $P = 0.002$), coinciding with the trends documented for *P.* cf. *isothrix*.

Aquatic Macroinvertebrates

During the course of this study, 17,433 aquatic macroinvertebrates from 67 taxa were collected and identified (Appendix Table 5). This data showed that aquatic macroinvertebrate communities differed between early summer (June – July) and late summer (August – September) (Fig. 6) (NMS final stress = 20.41; $p = 0.004$ for first three axes). In contrast, macroinvertebrate

communities appeared to be relatively similar among locations (MD, RC, VA). Within-year differences in community structure were related to the distribution of EPT taxa and changes in species richness. The HBI values were significantly higher in June and July when compared to August and September (mean values = 5.23 [SD = 0.27] and 4.24 [SD = 0.32], respectively). Percent abundance of EPT taxa was greater in August and September (average = 72%; SD = 0.09) than in June and July (average = 49%; SD = 0.09; one-way ANOVA, p = 0.0004). Taxa richness and diversity was also significantly greater in August and September (p = 0.006 and p = 0.02, respectively).

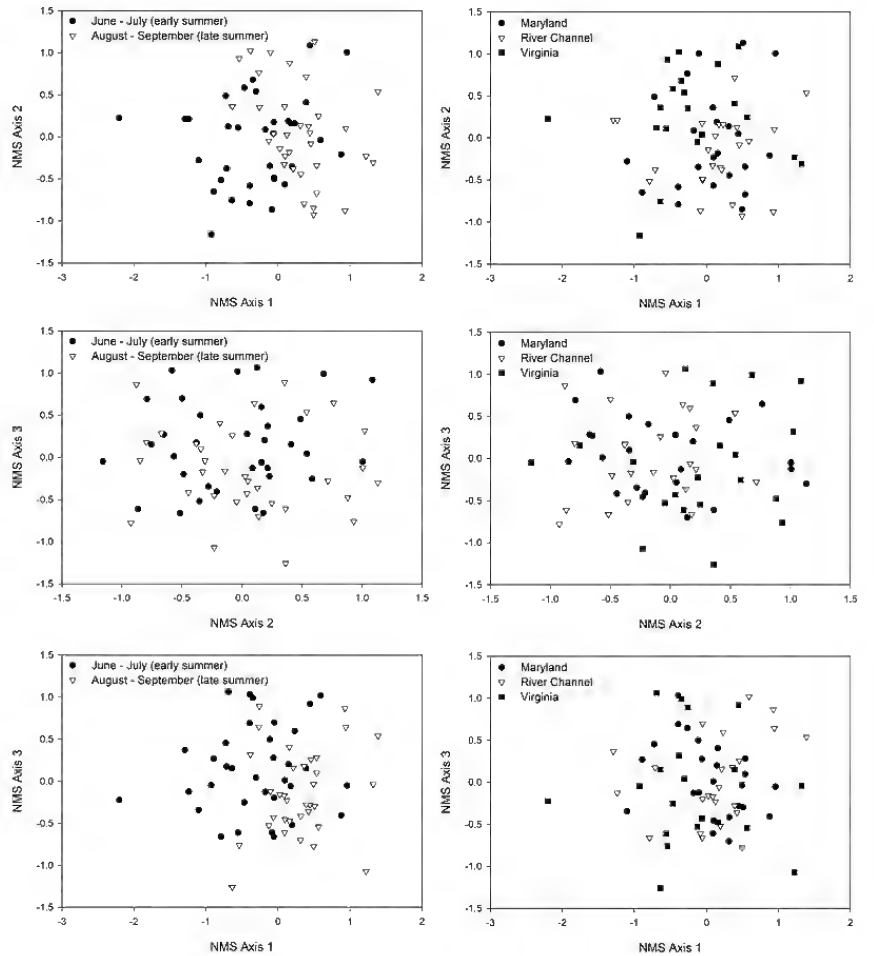

FIGURE 6. Plots of mean NMS scores (2013 and 2014). Plots on the left are temporal comparisons between early summer (June and July) versus late summer (August and September) and plots on the right are spatial (MD, RC, and VA). MD, RC, and VA signify Maryland, River Center, and Virginia sample locations, respectively.

Aquatic macroinvertebrate communities sampled during the *P.* cf. *isothrix* bloom in 2014 significantly differed from those in 2013, when no bloom occurred ($F = 2.64$, $p = 0.001$). This pattern was also evident in 2015. In 2015, macroinvertebrate communities collected from sites that experienced a *P.* cf. *isothrix* bloom differed significantly from those sites that did not experience blooms ($T = -2.88$, $A = 0.24$, $p = 0.02$). The %EPT and tolerant taxa differed between areas with and without benthic *P.* cf. *isothrix* blooms in 2015 (Table 4). In sites without *P.* cf. *isothrix*, the EPT taxa represented 86% of the community, whereas sites with *P.* cf. *isothrix* EPT taxa were 45% of the community. Similarly, locations without *P.* cf. *isothrix* were classified as "very good" by the HBI but only as "good" in locations with benthic *P.* cf. *isothrix* mats. When PC values indicated the presence of *P.* cf. *isothrix*, we observed proportionately more shredders than in areas without *P.* cf. *isothrix* (Fig. 7; $p = 0.01$).

TABLE 4. Summary of the benthic macroinvertebrate analyses for samples collected at the Brunswick, MD site in 2015. Data compares areas within the site that had a *Planktothrix* cf. *isothrix* bloom (Effect) to areas within the site that did not have an obvious *P.* cf. *isothrix* bloom (Control). Bold numbers indicate statistically significant ($p<0.05$) values. C = Control; E = Effect; BRRC – River Center station; BRMD – Maryland shoreline station.

Sample	Diversity	Equitability	Richness	Number	EPT%	Mean EPT%	HBI	Mean HBI
BRRC-C1	1.18	0.46	13	178	**88**	86%	**3.54 (very good)**	3.68 (very good)
BRMD-C1	1.79	0.72	12	152	**84**		**3.86 (very good)**	
BRMD-C2	1.99	0.72	16	98	**85**		**3.65 (very good)**	
BRMD-E1	1.68	0.64	14	180	43	45%	5.26 (good)	5.16 (good)
BRMD-E2	2.02	0.7	18	425	55		4.82 (good)	
BRRC-E1	1.94	0.7	16	163	36		5.4 (good)	

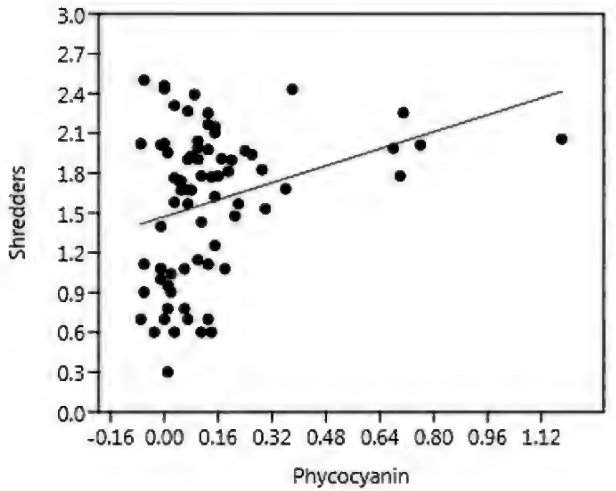

FIGURE 7. Bivariate linear regression model of the proportion of shredders (n = 5362; independent variable) and phycocyanin (n = 74; dependent variable), illustrating an increase in the shredders with an increase in phycocyanin. Data used for this analysis were collected throughout the entire sampling period (2013 through 2015).

DISCUSSION

The identification of a novel nuisance, and potentially toxic, cyanobacteria species in the lower non-tidal Potomac River was made using a combined morphological and phylogenetic analysis. This analysis identified the species as *Planktothrix* cf. *isothrix* based on the apical cell shape, cell dimensions, and a benthic to pelagic life cycle, as described by Komárek and Anagnostidis (2005). The Potomac River species is most closely related to *Planktothrix* sp. (strain UVFP1) from Spain. *Planktothrix* sp. (strain UVFP1) was reported by Camacho et al. (2005) to occur in shallow, well-oxygenated, low flow areas between Fuente Podrida spring and the Cabriel River. Similar to the *P*. cf. *isothrix* from the Potomac River, *Planktothrix* sp. (strain UVFP1) mats start to form after the spring floods subside when water levels are low, and temperatures are above 17ºC. Unfortunately, no morphological description for *Planktothrix* sp. (strain UVFP1) accompanied the report by Camacho et al. (2005). The phylogenetic relationship of the Cabriel River and Potomac River *Planktothrix*, as well as other benthic to pelagic *Planktothrix* species, should be further explored as cryptic speciation within the genus *Planktothrix* has been demonstrated by Lin et al. (2010) and Gaget et al. (2015) and documenting within-strain variability is crucial to understanding climate change driven responses (Burford et al., 2019). This appears to be the first record of *P*. cf *isothrix* in this region as only *P. agardhii* appears in species lists generated from freshwater surveys conducted by Marshall (2013; 2014). During the course of this study the benthic cyanobacteria mats appeared to be monospecific using light microscopy techniques; however, as stated by Bouma-Gregson et al. (2017), speciation within benthic habitats

has only recently become an area of focused efforts. Continued monitoring of the benthic cyanobacteria community in this region should be conducted to elucidate cryptic species or species shifts that may be the result of changes to flow regimes, temperature, light attenuation, and available nutrient pools.

Cyanotoxins are a focus of environmental monitoring programs world-wide (de Figueiredo et al., 2004; Paerl and Otten, 2013; Harke et al., 2016). These toxins are known to negatively impact aquatic macroinvertebrate abundance and community structure in lakes (Oberholster et al., 2008) and streams (Aboal et al., 2000; Wood et al., 2014b), and cyanotoxins, including microcystin, inhibit growth and cause high levels of mortality in aquatic macroinvertebrates (Ferrão-Filho and Kozlowsky-Suzuki, 2011). Within the Chesapeake Bay region, microcystins, produced by pelagic *Microcystis* blooms in the James River, have been found in emerging aquatic macroinvertebrates (i.e., EPT and chironomid genera) and in their avian (Moy et al., 2016), pelagic fish, shellfish, and blue crab (Wood et al., 2014a) consumers. Exposure to microcystins from pelagic cyanobacteria blooms have been shown to suppress fish health, behavior, development, and growth, and can cause endocrine system disruption (Ernst et al., 2006; Rogers et al., 2011; Marie et al., 2012). However, benthic cyanobacteria species are noted as different from their pelagic counterparts because of the dense and persistent nature of benthic blooms (Bouma-Gregson et al., 2017). In 2015, microcystin production by the Potomac River *P.* cf. *isothrix* populations found in mats collected at the Brunswick and White's Ferry sites was well below the US EPA Human Health Recreational Ambient Water Quality Criteria of 10 ppb (US EPA, 2016). Since 2017, limited sampling in the region suggests that *P.* cf. *isothrix* blooms have occurred annually (J. Wolny, unpublished data) and with increased toxin concentrations (C. Luckett and P. Brady, unpublished data). The threat posed to recreational users and drinking water sources from benthic cyanobacteria blooms is poorly understood and consequently guidelines are lacking (Wood et al., 2020). The intense use of the Potomac River for recreational activities (MD DNR, 2017), particularly during the summer period when *P.* cf. *isothrix* blooms, and as a source of drinking water, with > 65 water intakes servicing numerous water treatment facilities (Weidner, 2009), highlights the need for routine investigations of toxin-production by this region's benthic cyanobacteria community. Bioaccumulation and the environmental variables, such as light availability and nitrogen cycling, which influence microcystin production in *Planktothrix* species (Tonk et al., 2005; Chaffin et al., 2018), were not examined during this study but should be considered as part of future monitoring efforts to determine if toxin presence and concentration contributes to changes in the macroinvertebrate community structure that could lead to further disruption in the food web, as well as to assess human health risks.

In the absence of benthic cyanobacteria blooms in 2013, algal pigments were found uniformly distributed with increasing pigment concentrations from the study area's most upstream site to the most downstream site. However, when nuisance levels of *P.* cf. *isothrix* were present in 2014 and 2015 "hot spots" of elevated algal pigments were documented along the Maryland shoreline and river center. Observations made upstream of the study area indicate that conditions for algal blooms are present in a larger geographic area than the 31 km stretch of this study (data not shown). The "hot spot" or patchy spatial distribution of *P.* cf. *isothrix* might be explained by the nutrient contributions of tributaries and/or transition(s) of surrounding land use, in addition to

microhabitats within the river that could impact flow regime or light attenuation making conditions more or less suitable for *Planktothrix* proliferation. Our study area was too small to draw parallels with major riverine ecological models, but the significant differences between the occurrence of *P.* cf *isothrix* on the Maryland versus Virginia shorelines indicates more than just longitudinal river flow is a factor. Selckmann et al. (2018) reported similar findings when examining filamentous green algae (FGA) in an area just north of our Brunswick, Maryland site. In their study, Selckmann et al. (2018) found that FGA and an unidentified cyanobacteria bloomed on one shoreline but not the other despite there being no differences in water chemistry or habitat. The incorporation of riparian land use characteristics, climatic (specifically temperature) changes, and pulsed flood/flow events (man-made or natural) to the River Continuum Concept (Vannote et al., 1980) have been encouraged by Maiolini and Bruno (2007). Locally, Selckmann et al. (2018) and Shull et al. (2019) have demonstrated that water quality and biological communities in large rivers, such as the Potomac River, are subject not only to influence from shoreline land use but from tributaries as well and that increasing the spatial and temporal extent of samples, as well as the number of biological samples collected, in response to upstream tributaries increases the likelihood of distinguishing between impaired and non-impaired environments. An examination of a larger portion of the non-tidal Potomac River ecosystem along a broader temporal and spatial scale with these modeling concepts in mind should be conducted to better understand the environmental conditions that can promote benthic cyanobacteria blooms and changes in the aquatic macroinvertebrate community. The data collected during this geographically-limited survey showed that the local aquatic macroinvertebrate community was impacted by the presence of *P.* cf. *isothrix*. However, the extent to which both the aquatic macroinvertebrates and presence or toxicity of *P.* cf. *isothrix* was compounded by in-flow from the closely co-located Shenandoah River, which has a history of recurring algal blooms (Griggs et al., 2015), or from nutrient pools in the forested headwaters of the Potomac River was not examined.

Blooms of *P.* cf. *isothrix* proliferated in June and reached peak biomass from mid-to-late July when water temperatures were 21-28 °C, the optimal growth range reported for this species (Halstvedt et al., 2007). In addition to water temperature, blooms were also affected by stream flow. Stanfield (2018) determined that *P.* cf. *isothrix* blooms only occurred when flows were < 5,000 ft^3/sec. The genetically related *Planktothrix* sp. documented by Camacho et al. (2005) also had a preference for lower flow environments. The relationship between river flow and benthic cyanobacteria mat proliferation appears to be species-specific (see Suren et al., 2003b; Wood et al., 2017; Bouma-Gregson et al., 2019). In this region, climate change is predicted to create more intense precipitation and drought events, which will inevitably affect flow regime and the retention of N and P pools that may favor the growth of different benthic algal species (Bukaveckas et al., 2018; Reidmiller et al., 2018). This highlights the need for multi-faceted monitoring plans for ecosystems impacted by potentially harmful benthic algae and cyanobacteria.

Seasonal differences in the aquatic macroinvertebrate communities were evident throughout the study period and were largely attributed to cyclic patterns that were not directly influenced by the blooms of *P.* cf. *isothrix*. However, we found that benthic *P.* cf. *isothrix* blooms had a direct effect on the local aquatic macroinvertebrate communities as indicated with a decrease in %EPT and increased HBI designation. Freidman (2009) reported stable or improving trends in

aquatic macroinvertebrate communities from the non-tidal Potomac River. Our study suggests that impacts to the aquatic macroinvertebrate community due to the presence of *Planktothrix* may occur on a fine spatial scale, only noted when sample collections are designed to cover both within and outside of bloom patches. However, persistent alterations of the freshwater non-tidal Potomac River aquatic macroinvertebrate community, such as the decrease in %EPT and increase in HBI due to ecosystem disruptive cyanobacteria blooms and/or toxin production could have long-term, negative impacts for the recreational fisheries found here. While toxin production was low, natural mortality or emigration by aquatic macroinvertebrates in response to all aspects of the bloom could account for changes in the aquatic macroinvertebrate communities we documented. For the aquatic macroinvertebrates that remained associated with the *Planktothrix* mats, bioaccumulation of toxins could ultimately influence their own growth or have sub-lethal effects on their major predators, fishes, as has been documented with other cyanobacteria blooms by Wood et al. (2014a, b). A need for additional research regarding the total toxin production of this taxon, and any associated bioaccumulation and trophic transfer to river fauna exists and should be the focus of future efforts. Resource managers should account for the spatial and temporal distribution of benthic cyanobacteria mats when planning aquatic macroinvertebrate surveys to avoid bias and to accurately document changes that may be occurring within benthic flora and fauna communities.

Climate change and eutrophication are predicted to cause increases in the occurrence and toxicity of cyanobacteria blooms (Paerl and Huisman, 2009); future drought periods are likely to exacerbate the proliferation of cyanobacteria blooms and toxin production, which could degrade water quality and the aquatic macroinvertebrate community. If freshwater riverine systems change as predicted, then we encourage continued or even increased routine water quality monitoring coupled with detailed examinations of benthic flora and fauna in the interest of safeguarding human health and providing sound fisheries management.

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STATEMENT OF RESPONSIBILITIES

Joshua Henesy and John Mullican designed the study. All authors contributed to data collection and analysis. The first draft of the manuscript was written by Joshua Henesy and Jennifer Wolny and all authors provided edits to subsequent versions. All authors read and approved the final manuscript. Data used in this study is available at http://eyesonthebay.dnr.maryland.gov/eyesonthebay/DataInfo.cfm (accessed 10/25/2020).

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APPENDICES

APPENDIX TABLE 1. GSP coordinates for sampling locations on the Maryland shoreline (MD), river center (RC), and Virginia shoreline (VA) at Brunswick (BR), Maryland, Point of Rocks (PR), Maryland, and White's Ferry (WF), Virginia.

BRMD	39.31602N	-77.64895W
BRRC	39.31263N	-77.65401W
BRVA	39.31383N	-77.65172W
PRMD	39.27270N	-77.54175W
PRRC	39.27187N	-77.54287W
PRVA	39.27105N	-77.54327W
WFMD	39.15057N	-77.52048W
WFRC	39.15042N	-77.52261W
WFVA	39.15083N	-77.52390W

APPENDIX TABLE 2. Primer sequences used to examine *Planktothrix* from the non-tidal Potomac River.

Primer	Sequence (5' to 3')	Reference
CYA 106F	CGG ACG GGT GAG TAA CGC GTG A	Nübel et al. (1997)
CYA 781R (a)	GAC TAC TGG GGT ATC TAA TCC CAT T	Nübel et al. (1997)

APPENDIX TABLE 3. List of species, isolate designation, sampling locations and Genbank Accession numbers used in the sequence alignment analysis of *Planktothrix. Nostoc punctiforme* was used as outgroup. Sequence from this study is in bold.

Species	Isolate	Sampling location	Accession no.
P. agardhii	NIVA-CYA 11	Norway	AB045913
P. agardhii	NIVA-CYA 15	Norway	AB045923
P. agardhii	NIVA-CYA 313	Germany	AB045933
P. agardhii	NIVA-CYA 64/6	Norway	AB045941
P. agardhii	CCAP 1459/21	United Kingdom	AB045900
P. agardhii	NIVA-CYA	Norway	AB045932
P. cryptovaginata	NK1-11	China	MK625310
P. rubescens	NIVA-CYA	Denmark	AB045921
Planktothrix sp.	UVFP1	Spain	AJ630646
P. agardhii	PCC 9637	France	GQ351569
P. agardhii	CCAP 1460/133	Switzerland	HF678485
P. clathrata	PUPCCC	Northwestern Himalayas	KM384750
P. cf. *isothrix*	BRVA072915	Maryland, USA	**MH299785**
P. mougeotii	HAB626	China	FJ184391
P. paucivesiculata	PCC 8954	France	GQ351576
P. pseudagardhii	HAB2310	China	FJ184386
P. rubescens	SAG: 5.89	United Kingdom	KM019971
P. suspensa	NIES-3736	Japan	LC037449
P. tepida	PCC 9214	Central African Republic	GQ351566
Planktothrix sp.	VUW25	New Zealand	GQ451423
N. punctiforme	PCC 73102	Spain	NR074317

APPENDIX TABLE 4. Hilsenoff Biotic Index (HBI) Values and comments used for macroinvertebrate communities collected from the non-tidal Potomac from 2013-2015.

HBI Value Ranking	Comments
0.00 to 3.50 (excellent)	No apparent organic pollution
3.51 to 4.50 (very good)	Possible slight organic pollution
4.51 to 5.50 (good)	Some organic pollution
5.51 to 6.50 (fair)	Fairly significant organic pollution
6.51 to 7.50 (fairly poor)	Significant organic pollution
7.51 to 8.50 (poor)	Very significant organic pollution
8.51 to 10.00 (very poor)	Severe organic pollution

APPENDIX TABLE 5. Aquatic macroinvertebrate taxonomic list from samples collected from the lower, non-tidal Potomac River between June and September of 2013 through 2015. * indicates individuals where subsampling took place. ** signifies a lower taxonomic resolution (i.e., class instead of genera).

Order	Family (genera)	Total Abundance	Functional Feeding Group
Ephemeroptera	Potamanthidae (1)	4443	Filterer
	Baetidae (3)	1451	Collector
	Heptagenidae (4)	1063	Scraper
	Ephemerellidae (2)	12	Collector
	Ephemeridae (1)	21	Collector
	Caenidae (1)	244	Collector
	Tricorythidae (1)	1700	Collector
	Isonychiidae (1)	35	Filterer
	Polymitarcyidae (1)	1	Collector
Plecoptera	Perlidae (3)	35	Predator
Trichoptera	Hydropsychidae (4)	423	Filterer
	Leptoceridae (1)	12	Collector
	Brachycentridae (1)	28	Filterer
	Glossosomatidae (1)	44	Scraper
	Hydroptilidae (2)	11	Scraper
	Polycentropodidae (1)	6	Filterer
	Psychomyiidae (1)	11	Scraper
	Philopotamidae (1)	43	Filterer
*Amphipoda	Gammaridae (1)	5329	Shredder
Coleoptera	Elmidae (4)	1132	Scraper
	Haliplidae (1)	1	Shredder
	Dryopidae (1)	2	Scraper
	Psephenidae (2)	15	Scraper
	Gyrinidae (2)	2	Predator
Odonata	Coenagrionidae (3)	112	Predator
	Calopterygidae (1)	2	Predator
	Corduliidae (2)	5	Predator
	Gomphidae (4)	28	Predator
Megaloptera	Corydalidae (2)	9	Predator
	Sialidae (1)	23	Predator
Lepidoptera	Pyralidae (1)	6	Shredder
Diptera	Simuliidae (1)	123	Filterer
	Chironomidae (4)	800	Collector
	Tipulidae (2)	12	Shredder
	Empididae (1)	2	Predator
Platyhelminthes	Turbellaria (1)	172	Predator
**Class	**Oligochaeta (1)	45	Collector
**Class	**Hirudinea (1)	30	Predator

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Estimated 2020 CO_2 Emission Reductions in Virginia's Transportation Sector from COVID-19

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ABSTRACT

The initial lockdown phase of the COVID-19 pandemic presented an unfortunate opportunity to observe how abrupt, large-scale changes in traffic volume can reduce greenhouse gas emissions. This study explores how carbon dioxide (CO_2) emissions from Virginia's transportation sector may have been affected by the changes in activity stemming from COVID-19 to inform more carbon-neutral policies as the state recovers from the economic downfall. Emission savings were calculated by multiplying the percent change from 2019 to 2020 in traffic volume from the Virginia Department of Transportation with the business-as-usual 2020 U.S. Environmental Protection Agency estimate of CO_2 emissions for Virginia's transportation sector. We estimate Virginia's 2020 COVID-19 transportation CO_2 emissions reduction is around 15.0% (14.2 to 15.7%), with reduced passenger vehicle traffic making up the bulk of the inferred reduction. This study highlights the utility of reimagining our current transportation sector as a way to implement sustainable, state-level carbon reduction policies, such as the Clean Car Standards.

Keywords: Climate Change, CO2 Emissions, COVID-19, Transportation, Virginia

INTRODUCTION

Climate change is one of the most pressing environmental issues we are faced with today. Global temperatures have warmed by ~1.0°C since preindustrial times due to human activities, mostly as a result of burning fossil fuels (IPCC, 2014; USGCRP, 2017). The Paris Climate

Agreement has set an ambitious warming tolerance of 1.5°C in order to mitigate the worsening effects of climate change and prevent catastrophe (IPCC, 2018). To meet this target, global emissions need to be reduced by ~7.5% every year until net-zero emissions are met (Matthews and Caldeira, 2008; IPCC, 2018; UNEP, 2019). This will require systematic change driven by international, national, state, and even local policies to reduce emissions in such a stringent period (Rogelj et al., 2015; Davis et al., 2018).

Virginia emits the 35th highest amount of emissions per capita compared to the other states in the country (U.S. EIA, 2019). The state needs to eliminate its emissions by 2050 to aid the rest of the world in keeping global temperatures from warming more than 1.5°C. The benefits of doing so will extend beyond helping to reduce global temperature changes but will also help mitigate the local impacts related to climate change. Virginia is already experiencing more extremes in weather, with an increase in intense periods of precipitation (Allen and Allen, 2019) causing inland flooding, extended and intensified extreme heat events, and longer periods of prolonged drought (EPA, 2016). Sea level along Virginia's coast is rising at a much faster rate compared to the global average, increasing the frequency of tidal flooding and causing communities to be more vulnerable to storm surges (Ezer and Atkinson, 2015). The most important impact is how climate change is affecting human health. Summer temperature highs, in Richmond for example, are over several degrees hotter today than 50 years ago, and many places in the state are already experiencing more extreme heat days (Dahl et al., 2019). This leads to an increase in heat-related illnesses (Constible, 2018), particularly troublesome for our most marginalized communities who are exposed to higher summertime temperatures due to urban design (e.g., Hoffman et al., 2020). Runoff from heavy rains and intrusion of saltwater are polluting waterways, increasing water- and food-borne illnesses (Constible, 2018). Allergy season is longer (Anenberg et al., 2017) and there is an increase in tick- and mosquito-borne illnesses (Brownstein et al., 2005). Lastly, there is a strong correlation between fossil fuel emissions and air quality (WHO, 2006), which has been linked to asthma and many other respiratory conditions (e.g., Constible, 2018; Guarnieri and Balmes, 2014) as well as autism spectrum disorder (ASD), depression, and premature death (e.g., Penn et al., 2017; Calderón-Garcidueñas et al., 2015).

In April 2020, Virginia made progress towards committing to reducing greenhouse gas emissions by passing the Virginia Clean Economy Act (2020), which moves to shut down fossil fuel power plants, mandates for energy efficiency, and requires Virginia's major power suppliers, Dominion Energy and Appalachian Power, to be 100% carbon free by 2045 and 2050, respectively. Although the Virginia Clean Economy Act is a bold statement that illustrates the state's desire to adopt clean carbon-free energy sources, all while creating economic development through generating jobs, it leaves out Virginia's transportation sector. There is no specific policy to date that addresses emissions from the state's transportation sector, despite the fact that it contributes the majority of Virginia's overall carbon emissions (Fig. 1) and causes the formation of air pollutants that are harmful to public health.

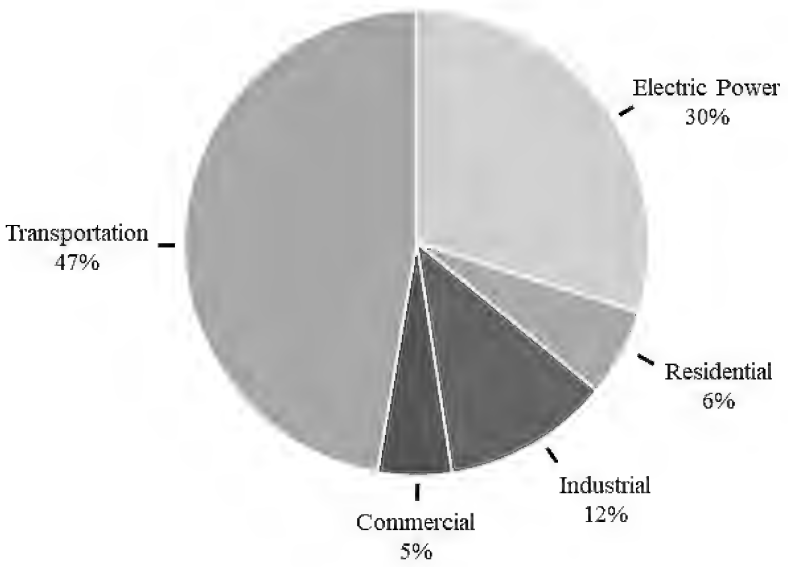

FIGURE 1. The five energy sectors of Virginia and their relative contributions to greenhouse gas emissions from 2010 to 2017. Data from the U.S. EIA.

In 2020, the global health crisis caused by the SARS-CoV-2 novel coronavirus, referred to here by its disease COVID-19, halted global economies as most developed nations went into confinement to prevent the spread of the virus (Sohrabi et al., 2020). This change in activity affected global energy demand in all sectors, especially air and ground transportation. Over the period of January 1−April 30, 2020, Le Quéré et al. (2020) estimated daily emission reductions on average of 26% for individual countries during their peak confinement, attributing most of the change to reduced ground transportation and energy demand from the power sector. Alongside global carbon emission reductions, remotely-sensed estimates of other pollutants saw concomitant and equally ephemeral reductions. In China, where the outbreak first occurred, nitrogen dioxide (NO_2), a major pollutant from gasoline-powered vehicles, dropped by almost 40%. Western Europe and the U.S. saw a 20-38% average decrease in satellite NO_2, compared to the same time period in 2019 (Bauwens et al., 2020). Monitoring stations on the ground in China reflected a 60% decrease in NO_2 as well as a 35% decrease in particulate matter smaller than 2.5 micrometers ($PM_{2.5}$), another major pollutant derived mostly from gasoline-powered vehicles (Shi and Brasseur, 2020). Although there have been many global and country-level studies of major pollutant reductions during the COVID-19 lockdowns, research into emission changes related to COVID-19 specific to the Commonwealth of Virginia has not yet been done.

Prior to the 2020 COVID-19 pandemic, Virginia's carbon dioxide (CO_2) emissions were decreasing slightly on average relative to the past, mostly due to the state's energy sector-driven transition from coal to natural gas (U.S. EIA, 2019). New natural gas power plants, though initially responsible for reducing the state's emissions, may ultimately hinder the state's carbon goals and make it more difficult to achieve net-zero emissions as outlined in the Virginia Clean Economy Act. In fact, some research suggests that no new carbon-emitting power plants should be commissioned if we are to meet the Paris Climate Agreement's goals globally (Tong et al., 2019).

During the early stages of the pandemic, Virginia similarly followed the rest of the world and the country when Governor Ralph Northam issued Executive Order 53 on March 23, 2020 restricting businesses and closing schools, and mandated Executive Order 55 on March 30, 2020 requiring people to stay at home. The state remained in confinement until most areas moved to Phase II on June 2, 2020 and then to Phase III on June 30, 2020, which allowed most businesses to reopen and operate under strict social distancing guidelines. This study aims to quantify Virginia's 2020 CO_2 emission savings stemming from the Commonwealth's reduced traffic volume, resulting from the COVID-19 stay-at-home orders. Our motivation stems from the observed traffic volume reductions around the world and the country, work focusing on large-scale greenhouse gas emissions, and inferred improvements in air quality. Because Virginia's transportation sector is its largest CO_2 emission source (U.S. EIA, 2019), the goal of this work is to highlight how the transportation sector in Virginia was impacted by COVID-19 policies to help inform the systematic changes needed to both reduce greenhouse gas emissions from transportation and improve local air quality.

DATA AND METHODS

Carbon emissions for the state of Virginia were divided into five sectors – transportation, electric power, industrial, commercial, and residential (Fig. 1) (U.S. EIA, 2019). The transportation sector, which accounted for almost half of Virginia's 2010-2017 average CO_2 emissions at 47%, consists of all vehicles whose primary purpose is to transport people or goods. According to the Virginia Department of Transportation's (VDOT) COVID-19 Traffic Trend Tool (Table 1), passenger vehicles accounted for 92% of the vehicles used in this sector while freight trucks made up only 8% in 2019.

TABLE 1. List of data sources used in the 2020 COVID-19 emission calculations

Source	Purpose	URL Link
VDOT - Virginia COVID-19 Traffic Trend Tool	Quantify reduction in traffic activity	https://public.tableau.com/profile/simona.babiceanu#!/vizhome/shared/GXW4B5FK3
EPA State CO_2 Emissions Projection Tool	Estimate BAU 2020 CO_2 emissions	https://www.epa.gov/statelocalenergy/download-state-inventory-and-projection-tool
U.S. EIA Energy Sales	Estimate emissions from the residential, commercial, and industrial sectors	https://www.eia.gov/electricity/data.php
U.S. Electric System Operating Data	Estimate emissions from the electric power sector	https://www.eia.gov/realtime_grid/

State-level annual CO_2 emission calculations from the U.S. Energy Information Administration (EIA) State Energy Data System (SEDS) are not published until at least two years after the annual energy data is published. Thus, real-time CO_2 emissions data were not available for Virginia at the time of writing. Changes in CO_2 emissions from the transportation sector were estimated using traffic counts collected by VDOT, provided through the Virginia COVID-19 Traffic Trend Tool. This tool measured traffic volumes, which were expressed as a percent difference from 2019, and distinguished between passenger vehicles and freight truck traffic. Thus, estimates are based off percent change from one year to the next. This approach does not account for if there were more electric and hybrid vehicles travelling during 2020 relative to the recent past. However, Virginia's current policy landscape makes it unlikely that there was a significant increase in these low or zero-emission vehicles on the road. The tool also does not differentiate between older, less efficient vehicles and newer, more efficient vehicles, which makes comparing emissions through time less accurate.

This 2020 COVID-19 transportation CO_2 estimate is based off the percent change in transportation activity from 2019. The Environmental Protection Agency's (EPA) projected transportation emissions for 2019 and business-as-usual (BAU) 2020 represent what emissions would have been if the pandemic lockdowns had never happened. The projected transportation emissions came from the EPA's CO_2 projection tool that estimates future state emissions based on historical energy trends through 2017 (Table 1). Because 2019 and 2020 transportation emissions were similar, BAU 2020 traffic volumes are assumed to be the same as 2019 and the 2019-2020 percent change in state-wide daily traffic volumes were averaged for each of the 12 months in the

year. These months were then averaged to obtain an annual percent change for 2020. The annual percent change was multiplied with the EPA's BAU 2020 transportation emissions projection to calculate the total annual CO_2 emissions reduction in $MMTCO_2E$, which is million metric tons of CO_2 standardized with other greenhouse gases. VDOT reported a 5% error in traffic counts, which were applied to estimate uncertainty bounds indicated in parentheses. The following equation summarizes the calculation used:

$$CO_2 \text{ emissions reduction } (MMTCO_2E) = \frac{\text{BAU 2020 projection } (MMTCO_2E) \times 2020 \text{ annual \% traffic volume change}}{100}$$

RESULTS

Traffic volumes were slightly higher in January and February of 2020 relative to 2019 (Fig. 2). After Governor Northam issued Executive Order 55, traffic volume was reduced from 17.2% in March to a peak of approximately 45.7% in April. Traffic volume then rebounded through September to a reduction of about 10% and stayed relatively steady for the remainder of 2020. The EPA's BAU Virginia transportation CO_2 emissions estimate for 2020 was 49.97 $MMTCO_2E$. Based on annual traffic volume reductions, it is estimated that transportation CO_2 emissions for 2020 were 42.48 $MMTCO_2E$ (42.11 to 42.86 $MMTCO_2E$) for Virginia due to COVID-19 lockdowns (Table 2). This is a reduction of 7.5 $MMTCO_2E$ (7.1 to 7.9 $MMTCO_2E$) or 15.0% (14.2 to 15.7%) from 2019. Transportation emissions in Virginia have not been this low since 1995 (Fig. 3).

This study finds passenger vehicle transport had the largest influence on emission reductions during the lockdown period. Vehicle type data from the Virginia COVID-19 Traffic Trend Tool revealed that passenger vehicle traffic, which accounted for 93% of vehicles on Virginia's roads in 2020, saw a 16.2% (15.4 to 17.1%) average reduction over the year, while freight truck traffic saw only a 1.94% (1.85 to 2.04%) average reduction for the same period (Table 3; Fig. 4).

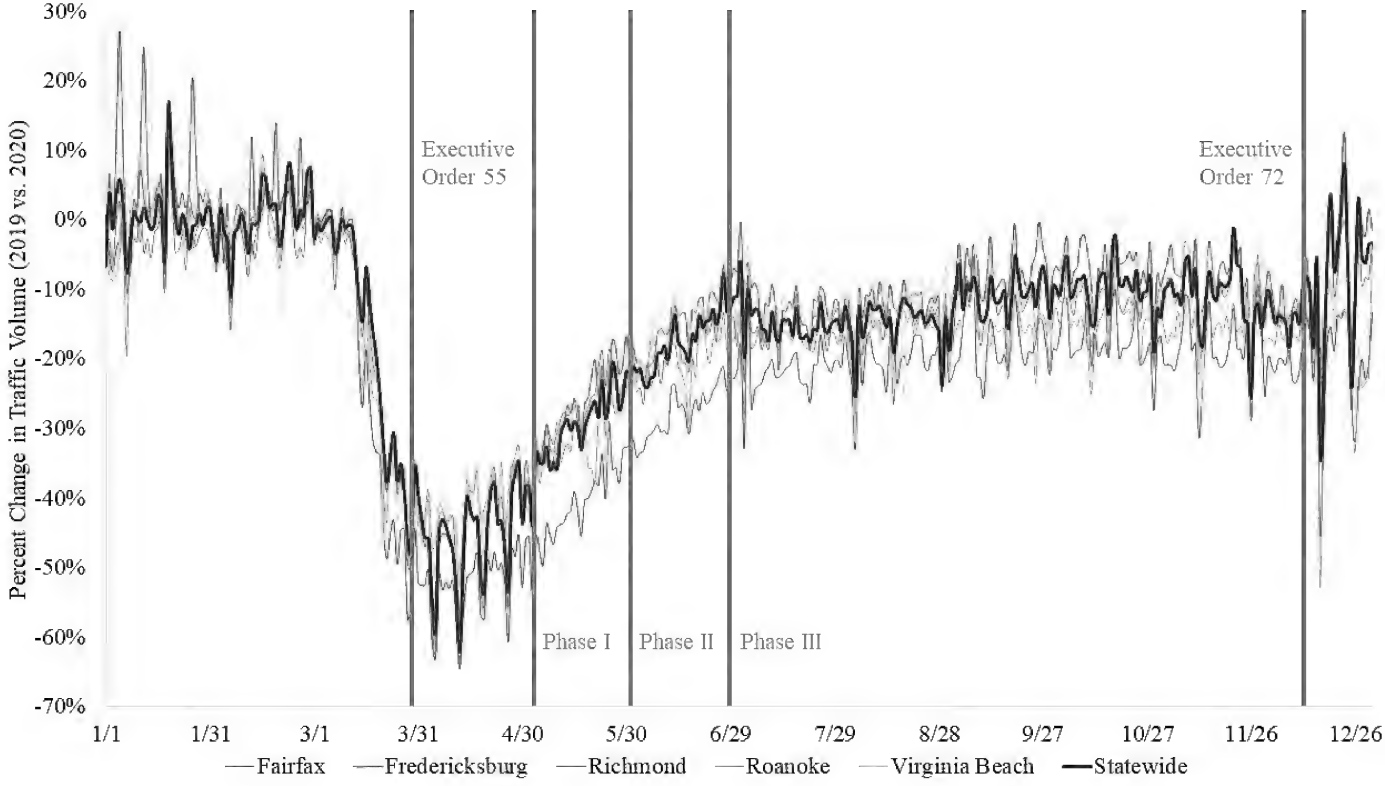

FIGURE 2. Percent change in transportation volume from 2019 for the state of Virginia (bold dark blue line) and select cities (thin blue-shaded lines). Gray vertical bars denote when Executive Order 55, the first stay-at-home order, was issued by the Governor (March 30, 2020), when the state subsequently entered Phase I (May 8, 2020), II (June 2, 2020), and III (June 30, 2020) of reopening, and Executive Order 72 (December 10, 2020), which introduced a "modified stay-at-home order" to slow the surging winter COVID-19 cases. Note: Fairfax and the City of Richmond delayed entering Phase I until May 29, 2020 and Phase II until June 12, 2020.

TABLE 2. Estimated 2020 CO_2 emissions reduction due to COVID-19 for Virginia's transportation sector

2020 BAU CO_2 emission estimate ($MMTCO_2E$)[a]	**Estimated annual change (% change)[b,c]**	**CO_2 reduction ($MMTCO_2E$)**	**2020 COVID-19 CO_2 emission estimate ($MMTCO_2E$)**
49.97	**-14.99** (-14.24 to -15.74)	-7.49 (-7.11 to -7.86)	**42.48** (42.11 to 42.86)
Estimated monthly change (% change)			
January	February	March	April
0.64 (0.61 to 0.68)	0.48 (0.46 to 0.51)	-17.46 (-16.59 to -18.34)	-45.74 (-43.45 to -48.03)
May	June	July	August
-29.96 (-28.46 to -31.46)	-17.16 (-16.30 to -18.02)	-14.71 (-13.79 to -15.44)	-14.83 (-14.09 to -15.57)
September	October	November	December
-10.51 (-9.98 to -11.03)	-9.81 (-9.32 to -10.30)	-10.84 (-10.30 to -11.38)	-9.95 (-9.46 to -10.45)

[a]Estimates from the EPA's CO_2 projection tool available at https://www.epa.gov/statelocalenergy/download-state-inventory-and-projection-tool
[b]Annual change is calculated by taking the average percent change from January through December.
[c]Error ranges of 5% as reported by VDOT are indicated in parentheses.

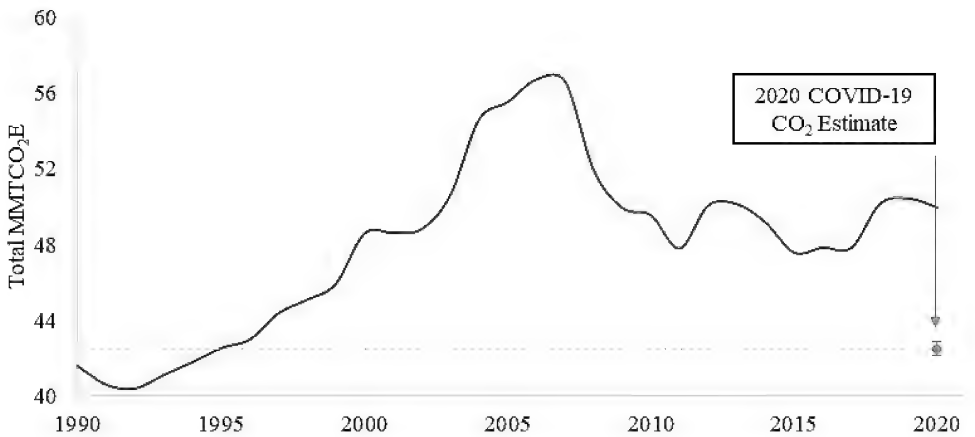

FIGURE 3. Virginia's transportation annual CO_2 emissions from 1990 to BAU 2020 (dark blue line) with the 2020 COVID-19 annual CO_2 estimate (red dot).

TABLE 3. Change in passenger vehicle and freight truck traffic volumes from 2019 to 2020

Passenger Vehicles		**Freight Trucks**	
Estimated monthly change (% change)			
January	July	January	July
0.42 (0.40 to 0.44)	-15.17 (-14.41 to -15.93)	-0.39 (-0.37 to -0.25)	-3.85 (-3.66 to -4.04)
February	August	February	August
0.93 (0.89 to 0.98)	-15.87 (-15.07 to -16.66)	-0.24 (-0.22 to -0.25)	0.63 (0.60 to 0.66)
March	September	March	September
-19.39 (-18.42 to -20.36)	-12.69 (-12.05 to -13.32)	-1.52 (-1.44 to -1.60)	0.55 (0.52 to 0.58)
April	October	April	October
-45.21 (-42.95 to -47.47)	-12.15 (-11.54 to -12.75)	-16.23 (-15.42 to -17.04)	3.79 (3.60 to 3.98)
May	November	May	November
-31.56 (-29.98 to -33.14)	-12.29 (-11.68 to -12.91)	-8.52 (-8.10 to -8.95)	5.62 (5.34 to 5.90)
June	December	June	December
-18.74 (-17.80 to -19.68)	-13.10 (-12.45 to -13.76)	-3.72 (-3.53 to -3.91)	0.58 (0.55 to 0.61)
Annual change (% change)[a]			
-16.23 (-15.42 to -17.05)		-1.94 (-1.85 to -2.04)	

[a]Annual change is calculated by taking the average percent change from January through December.

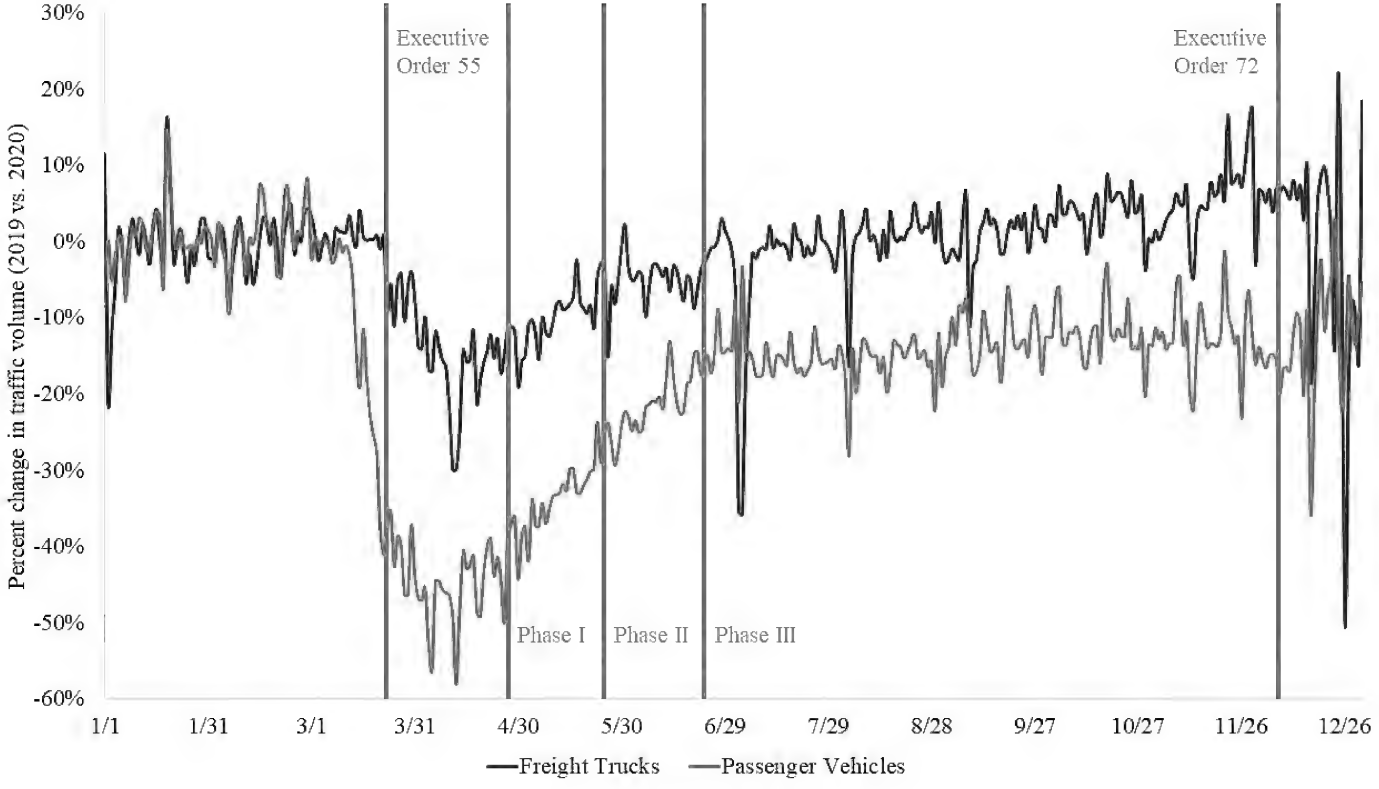

FIGURE 4. Percent change from 2019 in freight truck traffic (dark blue line) and passenger vehicle traffic (lighter blue line) for the state of Virginia in 2020.

DISCUSSION

Virginia's stay-at-home order had a considerable effect on the transportation sector, with the estimated CO_2 emission reductions equivalent to taking approximately 1.6 million passenger vehicles off the road for one year, according to the EPA's greenhouse gas equivalencies calculator. The COVID-19-related reduction in traffic volume in Virginia are similar to reductions in traffic across the U.S. and around the world. Our estimated 15.0% reduction for Virginia over the whole year is close to the Rhodium Group's U.S. estimate of 14.7%; however, their estimate is only based on January through October data (Larson et al., 2021). Virginia's peak monthly reduction in traffic volume in April of ~46% is similar to the estimated 50% reduction of average global traffic volumes during peak confinement (Le Quéré et al., 2020) and U.S. estimates of max reductions of 48% in early April (Schuman, 2020). Reduced traffic volumes were seen in all cities across the state but the more urbanized cities, particularly Northern Virginia, saw the largest reductions (Fig. 2). Additionally, overall ridership on public transit was significantly reduced during the confinement period (De Vos, 2020), further highlighting the impact that the stay-at-home order had on communities. Interestingly, the largest daily reductions occurred on weekends (Fig. 2), suggesting that people with more traditional working schedules were following Executive Order 55. However, while passenger vehicle traffic saw large reductions, there was little change in freight truck traffic volumes in Virginia, indicating that goods were still being ordered and delivered (Pishue, 2020).

Implications for reduced traffic extend far beyond reducing greenhouse gases, leading to quick and dramatic short-term improvements in air quality, as has been seen around the world in both satellite and ground measurements of NO_2 and $PM_{2.5}$ (Bauwens et al., 2020; Shi and Brasseur, 2020; Li et al., 2020). Even in Virginia, roadside ground measurements of NO_2 and $PM_{2.5}$ from the Virginia Department of Environmental Quality (DEQ) taken at Bryan Park in Richmond from mid-March to mid-April, when traffic volumes were most reduced, show reductions on average of 25% and 65%, respectively, during peak rush hour traffic on weekdays compared to the same period in previous years (Fig. 5). This suggests that locally, surface-level air quality near high-traffic road networks positively responded in real time to reductions in emissions driven by reductions in passenger traffic volumes at peak commuting hours (Kendrick et al., 2015). However, there is a complex, regional relationship between $PM_{2.5}$, NO_2, and weather, as seasonality in temperature, humidity, and wind can lead to inaccurate results. These air quality data must be normalized for these factors before drawing conclusions between these observed improvements in air quality and traffic volumes (Grange and Carslaw, 2019). Trends between weather-normalized ground-monitored air pollution and traffic volume over the last several years should be explored in more detail to fully quantify how the reduced traffic from COVID-19 affected air quality in several of Virginia's largest cities.

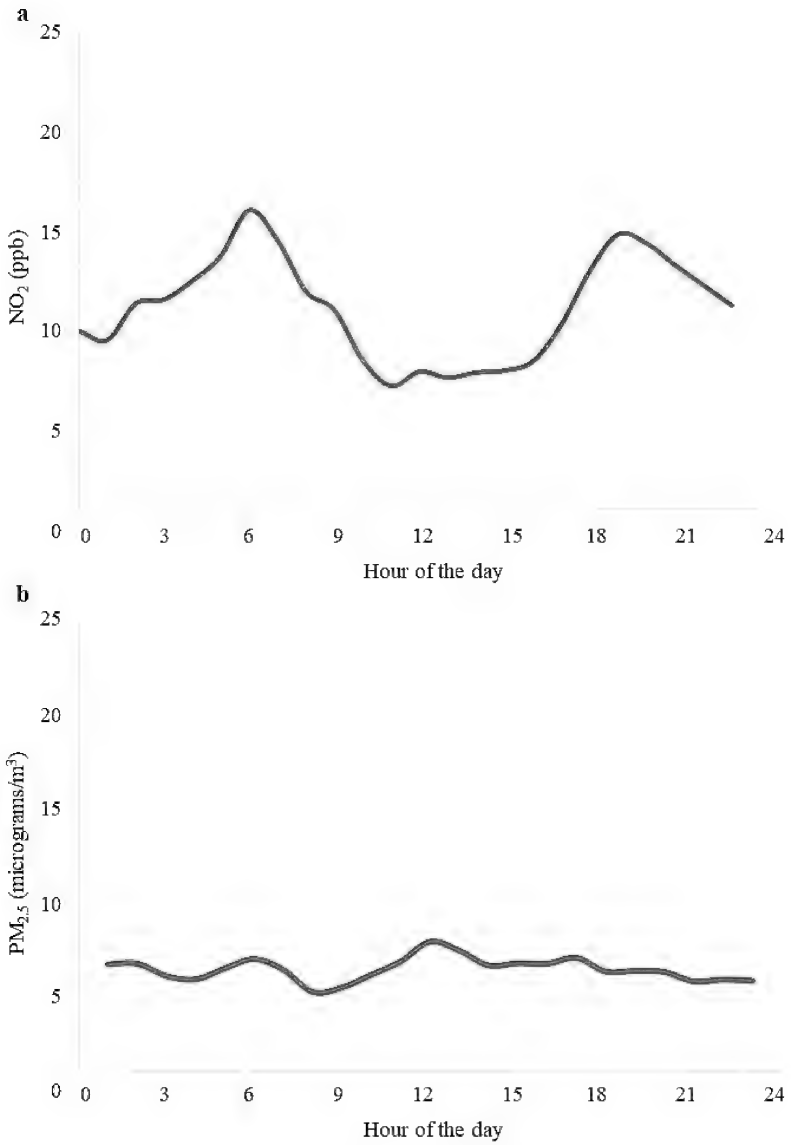

FIGURE 5. Hourly time series of A) NO_2 and B) $PM_{2.5}$ ground surface air quality measurements collected by DEQ from Bryan Park, Richmond, Virginia. Time series are hourly weekday averages from March 14 to April 14, 2020. The dark blue line denotes March 14 – April 14, 2020 and the gray lines denote the same time period for the years 2016 – 2019.

Poorer air quality related to proximity to burning fossil fuels and traffic emissions of $PM_{2.5}$ has major implications for human health (WHO, 2006), and those from lower income communities and communities of color are consistently shown to be disproportionately exposed to these stressors (e.g., Hooper and Kaufman, 2017). Air pollution from NO_2 not only increases respiratory problems, such as asthma, wheezing, and coughing, but also reacts in the atmosphere to form ground-level ozone (Sillman, 1999), leading to additional health problems related to the airway and lungs (WHO, 2006). Particulate matter, especially $PM_{2.5}$, accumulates deep inside the lungs, further aggravating respiratory symptoms, but can also lead to cardiac problems, lung disease, and even premature death (WHO, 2006; Anderson et al., 2012). Additionally, new research has shown that localities with poorer air quality are at greater risk for contracting (due to socioeconomics), suffering more severe cases, and/or dying from COVID-19 (Wu et al., 2020; Zoran et al., 2020; Hendryx and Luo, 2020; Conticini et al., 2020), as COVID-19 is a respiratory disease (Sohrabi et al., 2020). These connections between improved air quality and health related to reduced COVID-19 fossil fuel emissions are only beginning as researchers untangle the complex relationships between the two.

A 15.0% reduction in traffic emissions for the whole year is considerable. However, if it is the only energy sector that saw significant COVID-19-related reductions, the state's overall emissions for 2020 would only be reduced by around 7.45% (7.08 to 7.83%), saving 7.49 $MMTCO_2E$ (7.11 to 7.86 $MMTCO_2E$). However, studies suggest that other energy sectors experienced changes related to COVID-19 as well (Larson et al., 2021; Le Quéré et al., 2020). The Rhodium Group study covering the whole U.S. from January to October found 10.3% and 7.0% reductions for the electric power and industrial sectors as well as a 6.2% reduction for building emissions (Larson et al., 2021). If we assume that Virginia's other energy sectors experienced similar emission changes to what has been estimated more broadly by the Rhodium Group, the state would expect a reduction of 11.8 $MMTCO_2E$ (11.4 to 12.2 $MMTCO_2E$), or ~11.7%, assuming the EPA's 2020 pre-COVID-19 emissions estimates as BAU (Table 1). We acknowledge that Virginia's emissions behavior may not be reflected in the patterns of the U.S. more generally; thus, we explored state-level trends in energy sales and energy demand from the U.S. EIA from March through June, the peak of the confinement period (Table 1). We suggest that Virginia's total emission reductions estimated here (~8.54% decrease from 2019), while based on broader geographical patterns, may actually be more limited than the U.S.-wide emission reduction estimates. If this lower-end estimate is true, this equates to an emission savings of ~8.58 $MMTCO_2E$ and puts Virginia's annual CO_2 emissions for 2020 below 1990 levels. However, the available data for the other energy sectors contains different spatial scales and does not account for emission coefficients, which creates additional uncertainty. Thus, we caution that these are only a range of estimates that puts into perspective how widespread public activity changes can significantly impact carbon emissions at the state scale.

RECOMMENDATIONS

This study indicates that Virginia's transportation sector carbon emissions can respond rapidly to policy changes. The Virginia Conservation Network (VCN) has outlined several policies

that Virginia legislators could take to reduce emissions and air pollution from passenger vehicles (available at: http://www.vcnva.org/curbing-vehicle-pollution/), and they include two pathways. One path would aim to shift the transportation system away from depending so heavily on passenger vehicle transportation and its related infrastructure. This could include incentives for teleworking or ride sharing, multi-year investments in transit expansion between urban areas and further into rural areas, smarter planning of communities around reliable and frequent public transportation hubs, better trail and protected urban systems for biking and walking, and more research – including public health impacts – on the emission-related effects of new road projects like lane expansions before they are approved and/or implemented. The second pathway would be to improve fuel emission standards while transitioning Virginia's electrical grid to accommodate more electric vehicles. This second pathway – which would largely swap the current dependency on fossil-fueled cars (and the accompanying sprawl of population centers) with electric ones – would do little to realize the additional environmental, public health, and socioeconomic co-benefits of living in more connected, less sprawling, and largely healthier communities (Stone, 2008).

Overall, Virginia has made limited progress toward reducing transportation sector carbon emissions over the last decade. However, many Virginia cities are using incentives, such as Power Purchase Agreements, to add solar panels to school and government buildings, which require little to no money invested but come with large energy savings that can then be invested back into the cities and schools. This model could also support more efficient transportation system changes, such as implementing more electric vehicle (EV) charging stations across the state to increase driving range. Policies supporting renewable energy have shown to reduce greenhouse gas emissions (Le Quéré et al., 2019) and therefore, it is important for Virginia to continue improving these types of policies while also addressing transportation.

There are arguably more detrimental transportation policies than environmentally friendly ones in the state right now. Consumer Reports analyst Jeff Plungis (2019) explains that existing and proposed EV fees aimed at being equivalent to gas taxes are up to 61% higher than those for gas-powered vehicles in Virginia. However, there are bills currently on the table to improve Virginia's transportation policies. The Virginia Clean Car Standards bill would set two new requirements. The first would be the Low Emissions Vehicle (LEV) standard, which would require manufacturers to reduce emissions for the new gas-consuming vehicles they seek to sell in Virginia markets. The Zero Emissions Vehicle (ZEV) standard would add more electric and hybrid vehicles to the pool of purchasable vehicles in Virginia each year. Most car manufacturers send the majority of their EVs to states that already have Clean Car Standards in place, such as Maryland (Generation180, 2020), leaving little inventory for Virginia-based consumers. As the impacts from climate change and air pollution disproportionately affect historically marginalized communities, adopting policies like the Clean Car Standards that control CO_2 emissions and air quality is one critical step forwards for environmental equity and social justice as well (e.g., Clark et al., 2014). While these proposed standards are a daring start, further and decisive action will still be required to curb state emissions from the transportation sector.

CONCLUSION

This study estimates that Virginia's 2020 transportation CO_2 emissions were reduced by 15.0% (14.2 to 15.7%) from 2019 levels because of the abrupt traffic volume decrease stemming from COVID-19 public health interventions. Of course, strict public confinement is not a permanent solution to addressing transportation emissions and these changes do not reflect the systematic change in transportation types that would ultimately drive down emissions permanently each year. However, this study shows that the transportation sector, which makes up almost 50% of Virginia's CO_2 emissions on average, is responsive to policies that mandate transportation changes. Thus, transportation should be considered as a primary target for reducing Virginia's carbon emissions as well as further improving air quality. Even as new Federal leadership strengthens or reinstates environmental policies, state and local policies remain critical in addressing the root cause of present-day climate change. Although promising progress has been made in the electric power sector with Virginia's Clean Economy Act, policies for reducing transportation-related emissions are currently lacking. We recommend the state, alongside cities and counties, work with proposed VCN policy measures to lay a path towards attainable goals to reduce greenhouse gas emissions from the transportation sector. This will be critical to 1) help the country meet its commitment to the IPCC 1.5°C warming target to limit the economical, ecological, and human health impacts from climate change, and 2) better the people of Virginia's well-being through improved air quality, more community-oriented infrastructure, and improved economic and social equality. We acknowledge that the COVID-19 pandemic has been and continues to be disastrous for communities and families, especially those that have been historically marginalized through discriminatory public policy. We do not assert here that the pandemic has yielded positive effects. Rather, this work highlights that COVID-19 has provided a momentary glimpse at geophysical phenomena, useful toward envisioning a fairer future where reductions in emissions and improvements in air quality are permanent in Virginia as well as globally. Our findings underscore the fact that Virginia must support robust and equitable policies as the state recovers from the pandemic to reduce greenhouse gas emissions, especially from the transportation sector.

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Statement of Responsibility: P. Grothe (corresponding author) and J. Hoffman formulated the project. E. Rakes collected the traffic, energy, and emission data. E. Rakes and P. Grothe analyzed

the data and wrote the manuscript. J. Hoffman collected and analyzed the air quality data and provided feedback on the manuscript.

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